

**METHODS FOR IDENTIFYING DRUG TARGETS
BASED ON GENOMIC SEQUENCE DATA**

Background of the Invention

Related Applications

This application is a continuation of Application Number 09/243,022, filed February 2, 1999.

Field of the Invention

This invention relates to methods for identifying drug targets based on genomic sequence data. More specifically, this invention relates to systems and methods for determining suitable molecular targets for the directed development of antimicrobial agents.

Description of the Related Art

Infectious disease is on a rapid rise and threatens to regain its status as a major health problem. Prior to the discovery of antibiotics in the 1930s, infectious disease was a major cause of death. Further discoveries, development, and mass production of antibiotics throughout the 1940s and 1950s dramatically reduced deaths from microbial infections to a level where they effectively no longer represented a major threat in developed countries.

Over the years antibiotics have been liberally prescribed and the strong selection pressure that this represents has led to the emergence of antibiotic resistant strains of many serious human pathogens. In some cases selected antibiotics, such as vancomycin, literally represent the last line of defense against certain pathogenic bacteria such as *Staphylococcus*. The possibility for staphylococci to acquire vancomycin resistance through exchange of genetic material with enterococci, which are commonly resistant to vancomycin, is a serious issue of concern to health care specialists. The pharmaceutical industry continues its search for new antimicrobial compounds, which is a lengthy and tedious, but very important process. The rate of development and introduction of new antibiotics appears to no longer be able to keep up with the evolution of new antibiotic resistant organisms. The rapid emergence of antibiotic resistant organisms threatens to lead to a serious widespread health care concern.

The basis of antimicrobial chemotherapy is to selectively kill the microbe with minimal, and ideally no, harm to normal human cells and tissues. Therefore, ideal targets for antibacterial action are biochemical processes that are unique to bacteria, or those that are sufficiently different from the corresponding mammalian processes to allow acceptable discrimination between the two. For effective antibiotic action it is clear that a vital target must exist in the bacterial cell and that the antibiotic be delivered to the target in an active form. Therefore resistance to an antibiotic can arise from: (i) chemical destruction or inactivation of the antibiotic; (ii) alteration of the target site to reduce or eliminate effective antibiotic binding; (iii) blocking antibiotic entry into the cell, or rapid removal from the cell after entry; and (iv) replacing the metabolic step inhibited by the antibiotic.

Thus, it is time to fundamentally re-examine the philosophy of microbial killing strategies and develop new paradigms. One such paradigm is a holistic view of cellular metabolism. The identification of "sensitive" metabolic steps in attaining the necessary metabolic flux distributions to support growth and survival that can be attacked to weaken or destroy a microbe, need not be localized to a single biochemical reaction or cellular process. Rather, different cellular targets that need not be intimately related in the metabolic topology could be chosen based on the concerted effect the loss of each of these functions would have on metabolism.

A similar strategy with viral infections has recently proved successful. It has been shown that "cocktails" of different drugs that target different biochemical processes provide enhanced success in fighting against HIV infection. Such a paradigm shift is possible only if the necessary biological information as well as appropriate methods of rational analysis are available. Recent advances in the field of genomics and bioinformatics, in addition to mathematical modeling, offer the possibility to realize this approach.

At present, the field of microbial genetics is entering a new era where the genomes of several microorganisms are being completely sequenced. It is expected that in a decade, or so, the nucleotide sequences of the genomes of all the major human pathogens will be completely determined. The sequencing of the genomes of pathogens such as *Haemophilus influenzae* has allowed researchers to compare the homology of proteins encoded by the open reading frames (ORFs) with those of *Escherichia coli*, resulting in valuable insight into the *H. influenzae*

metabolic features. Similar analyses, such as those performed with *H. influenzae*, will provide details of metabolism spanning the hierarchy of metabolic regulation from bacterial genomes to phenotypes.

These developments provide exciting new opportunities to carry out conceptual experiments *in silico* to analyze different aspects of microbial metabolism and its regulation. Further, the synthesis of whole-cell models is made possible. Such models can account for each and every single metabolic reaction and thus enable the analysis of their role in overall cell function. To implement such analysis, however, a mathematical modeling and simulation framework is needed which can incorporate the extensive metabolic detail but still retain computational tractability. Fortunately, rigorous and tractable mathematical methods have been developed for the required systems analysis of metabolism.

A mathematical approach that is well suited to account for genomic detail and avoid reliance on kinetic complexity has been developed based on well-known stoichiometry of metabolic reactions. This approach is based on metabolic flux balancing in a metabolic steady state. The history of flux balance models for metabolic analyses is relatively short. It has been applied to metabolic networks, and the study of adipocyte metabolism. Acetate secretion from *E. coli* under ATP maximization conditions and ethanol secretion by yeast have also been investigated using this approach.

The complete sequencing of a bacterial genome and ORF assignment provides the information needed to determine the relevant metabolic reactions that constitute metabolism in a particular organism. Thus a flux-balance model can be formulated and several metabolic analyses can be performed to extract metabolic characteristics for a particular organism. The flux balance approach can be easily applied to systematically simulate the effect of single, as well as multiple, gene deletions. This analysis will provide a list of sensitive enzymes that could be potential antimicrobial targets.

The need to consider a new paradigm for dealing with the emerging problem of antibiotic resistant pathogens is a problem of vital importance. The route towards the design of new antimicrobial agents must proceed along directions that are different from those of the past. The rapid growth in bioinformatics has provided a wealth of biochemical and genetic information that can be used to synthesize complete representations of cellular metabolism. These models can

be analyzed with relative computational ease through flux-balance models and visual computing techniques. The ability to analyze the global metabolic network and understand the robustness and sensitivity of its regulation under various growth conditions offers promise in developing novel methods of antimicrobial chemotherapy.

In one example, Pramanik et al. described a stoichiometric model of *E. coli* metabolism using flux-balance modeling techniques (*Stoichiometric Model of Escherichia coli Metabolism: Incorporation of Growth-Rate Dependent Biomass Composition and Mechanistic Energy Requirements, Biotechnology and Bioengineering*, Vol. 56, No. 4, November 20, 1997). However, the analytical methods described by Pramanik, et al. can only be used for situations in which biochemical knowledge exists for the reactions occurring within an organism. Pramanik, et al. produced a metabolic model of metabolism for *E. coli* based on biochemical information rather than genomic data since the metabolic genes and related reactions for *E. coli* had already been well studied and characterized. Thus, this method is inapplicable to determining a metabolic model for organisms for which little or no biochemical information on metabolic enzymes and genes is known. It can be envisioned that in the future the only information we may have regarding an emerging pathogen is its genomic sequence. What is needed in the art is a system and method for determining and analyzing the entire metabolic network of organisms whose metabolic reactions have not yet been determined from biochemical assays. The present invention provides such a system.

Summary of the Invention

This invention relates to constructing metabolic genotypes and genome specific stoichiometric matrices from genome annotation data. The functions of the metabolic genes in the target organism are determined by homology searches against databases of genes from similar organisms. Once a potential function is assigned to each metabolic gene of the target organism, the resulting data is analyzed. In one embodiment, each gene is subjected to a flux-balance analysis to assess the effects of genetic deletions on the ability of the target organism to produce essential biomolecules necessary for its growth. Thus, the invention provides a high-throughput computational method to screen for genetic deletions which adversely affect the growth capabilities of fully sequenced organisms.

Embodiments of this invention also provide a computational, as opposed to an experimental, method for the rapid screening of genes and their gene products as potential drug targets to inhibit an organism's growth. This invention utilizes the genome sequence, the annotation data, and the biomass requirements of an organism to construct genomically complete metabolic genotypes and genome-specific stoichiometric matrices. These stoichiometric matrices are analyzed using a flux-balance analysis. This invention describes how to assess the affects of genetic deletions on the fitness and productive capabilities of an organism under given environmental and genetic conditions.

Construction of a genome-specific stoichiometric matrix from genomic annotation data is illustrated along with applying flux-balance analysis to study the properties of the stoichiometric matrix, and hence the metabolic genotype of the organism. By limiting the constraints on various fluxes and altering the environmental inputs to the metabolic network, genetic deletions may be analyzed for their affects on growth. This invention is embodied in a software application that can be used to create the stoichiometric matrix for a fully sequenced and annotated genome. Additionally, the software application can be used to further analyze and manipulate the network so as to predict the ability of an organism to produce biomolecules necessary for growth, thus, essentially simulating a genetic deletion.

Brief Description of the Drawings

Figure 1 is a flow diagram illustrating one procedure for creating metabolic genotypes from genomic sequence data for any organism.

Figure 2 is a flow diagram illustrating one procedure for producing *in silico* microbial strains from the metabolic genotypes created by the method of Figure 1, along with additional biochemical and microbiological data.

Figure 3 is a graph illustrating a prediction of genome scale shifts in transcription. The graph shows the different phases of the metabolic response to varying oxygen availability, starting from completely aerobic to completely anaerobic in *E. coli*. The predicted changes in expression pattern between phases II and V are indicated.

Detailed Description of the Invention

This invention relates to systems and methods for utilizing genome annotation data to construct a stoichiometric matrix representing most of all of the metabolic reactions that occur within an organism. Using these systems and methods, the properties of this matrix can be studied under conditions simulating genetic deletions in order to predict the affect of a particular gene on the fitness of the organism. Moreover, genes that are vital to the growth of an organism can be found by selectively removing various genes from the stoichiometric matrix and thereafter analyzing whether an organism with this genetic makeup could survive. Analysis of these lethal genetic mutations is useful for identifying potential genetic targets for anti-microbial drugs.

It should be noted that the systems and methods described herein can be implemented on any conventional host computer system, such as those based on Intel® microprocessors and running Microsoft Windows operating systems. Other systems, such as those using the UNIX or LINUX operating system and based on IBM®, DEC® or Motorola® microprocessors are also contemplated. The systems and methods described herein can also be implemented to run on client-server systems and wide-area networks, such as the Internet.

Software to implement the system can be written in any well-known computer language, such as Java, C, C++, Visual Basic, FORTRAN or COBOL and compiled using any well-known compatible compiler.

The software of the invention normally runs from instructions stored in a memory on the host computer system. Such a memory can be a hard disk, Random Access Memory, Read Only Memory and Flash Memory. Other types of memories are also contemplated to function within the scope of the invention.

A process 10 for producing metabolic genotypes from an organism is shown in Figure 1. Beginning at a start state 12, the process 10 then moves to a state 14 to obtain the genomic DNA sequence of an organism. The nucleotide sequence of the genomic DNA can be rapidly determined for an organism with a genome size on the order of a few million base pairs. One method for obtaining the nucleotide sequences in a genome is through commercial gene databases. Many gene sequences are available on-line through a number of sites (see, for example, www.tigr.org) and can easily be downloaded from the Internet. Currently, there are 16

microbial genomes that have been fully sequenced and are publicly available, with countless others held in proprietary databases. It is expected that a number of other organisms, including pathogenic organisms will be found in nature for which little experimental information, except for its genome sequence, will be available.

Once the nucleotide sequence of the entire genomic DNA in the target organism has been obtained at state 14, the coding regions, also known as open reading frames, are determined at a state 16. Using existing computer algorithms, the location of open reading frames that encode genes from within the genome can be determined. For example, to identify the proper location, strand, and reading frame of an open reading frame one can perform a gene search by signal (promoters, ribosomal binding sites, etc.) or by content (positional base frequencies, codon preference). Computer programs for determining open reading frames are available, for example, by the University of Wisconsin Genetics Computer Group and the National Center for Biotechnology Information.

After the location of the open reading frames have been determined at state 16, the process 10 moves to state 18 to assign a function to the protein encoded by the open reading frame. The discovery that an open reading frame or gene has sequence homology to a gene coding for a protein of known function, or family of proteins of known function, can provide the first clues about the gene and it's related protein's function. After the locations of the open reading frames have been determined in the genomic DNA from the target organism, well-established algorithms (i.e. the Basic Local Alignment Search Tool (BLAST) and the FAST family of programs can be used to determine the extent of similarity between a given sequence and gene/protein sequences deposited in worldwide genetic databases. If a coding region from a gene in the target organism is homologous to a gene within one of the sequence databases, the open reading frame is assigned a function similar to the homologously matched gene. Thus, the functions of nearly the entire gene complement or genotype of an organism can be determined so long as homologous genes have already been discovered.

All of the genes involved in metabolic reactions and functions in a cell comprise only a subset of the genotype. This subset of genes is referred to as the metabolic genotype of a particular organism. Thus, the metabolic genotype of an organism includes most or all of the genes involved in the organism's metabolism. The gene products produced from the set of

metabolic genes in the metabolic genotype carry out all or most of the enzymatic reactions and transport reactions known to occur within the target organism as determined from the genomic sequence.

To begin the selection of this subset of genes, one can simply search through the list of functional gene assignments from state 18 to find genes involved in cellular metabolism. This would include genes involved in central metabolism, amino acid metabolism, nucleotide metabolism, fatty acid and lipid metabolism, carbohydrate assimilation, vitamin and cofactor biosynthesis, energy and redox generation, etc. This subset is generated at a state 20. The process 10 of determining metabolic genotype of the target organism from genomic data then terminates at an end stage 22.

Referring now to Figure 2, the process 50 of producing a computer model of an organism. This process is also known as producing *in silico* microbial strains. The process 50 begins at a start state 52 (same as end state 22 of process 10) and then moves to a state 54 wherein biochemical information is gathered for the reactions performed by each metabolic gene product for each of the genes in the metabolic genotype determined from process 10.

For each gene in the metabolic genotype, the substrates and products, as well as the stoichiometry of any and all reactions performed by the gene product of each gene can be determined by reference to the biochemical literature. This includes information regarding the irreversible or reversible nature of the reactions. The stoichiometry of each reaction provides the molecular ratios in which reactants are converted into products.

Potentially, there may still remain a few reactions in cellular metabolism which are known to occur from *in vitro* assays and experimental data. These would include well characterized reactions for which a gene or protein has yet to be identified, or was unidentified from the genome sequencing and functional assignment of state 14 and 18. This would also include the transport of metabolites into or out of the cell by uncharacterized genes related to transport. Thus one reason for the missing gene information may be due to a lack of characterization of the actual gene that performs a known biochemical conversion. Therefore upon careful review of existing biochemical literature and available experimental data, additional metabolic reactions can be added to the list of metabolic reactions determined from

the metabolic genotype from state 54 at a state 56. This would include information regarding the substrates, products, reversibility/irreversibility, and stoichiometry of the reactions.

All of the information obtained at states 54 and 56 regarding reactions and their stoichiometry can be represented in a matrix format typically referred to as a stoichiometric matrix. Each column in the matrix corresponds to a given reaction or flux, and each row corresponds to the different metabolites involved in the given reaction/flux. Reversible reactions may either be represented as one reaction that operates in both the forward and reverse direction or be decomposed into one forward reaction and one backward reaction in which case all fluxes can only take on positive values. Thus, a given position in the matrix describes the stoichiometric participation of a metabolite (listed in the given row) in a particular flux of interest (listed in the given column). Together all of the columns of the genome specific stoichiometric matrix represent all of the chemical conversions and cellular transport processes that are determined to be present in the organism. This includes all internal fluxes and so called exchange fluxes operating within the metabolic network. Thus, the process 50 moves to a state 58 in order to formulate all of the cellular reactions together in a genome specific stoichiometric matrix. The resulting genome specific stoichiometric matrix is a fundamental representation of a genetically and biochemically defined genotype.

After the genome specific stoichiometric matrix is defined at state 58, the metabolic demands placed on the organism are calculated. The metabolic demands can be readily determined from the dry weight composition of the cell. In the case of well-studied organisms such as *Escherichia coli* and *Bacillus subtilis*, the dry weight composition is available in the published literature. However, in some cases it will be necessary to experimentally determine the dry weight composition of the cell for the organism in question. This can be accomplished with varying degrees of accuracy. The first attempt would measure the RNA, DNA, protein, and lipid fractions of the cell. A more detailed analysis would also provide the specific fraction of nucleotides, amino acids, etc. The process 50 moves to state 60 for the determination of the biomass composition of the target organism.

The process 50 then moves to state 62 to perform several experiments that determine the uptake rates and maintenance requirements for the organism. Microbiological experiments can be carried out to determine the uptake rates for many of the metabolites that are transported into

the cell. The uptake rate is determined by measuring the depletion of the substrate from the growth media. The measurement of the biomass at each point is also required, in order to determine the uptake rate per unit biomass. The maintenance requirements can be determined from a chemostat experiment. The glucose uptake rate is plotted versus the growth rate, and the y-intercept is interpreted as the non-growth associated maintenance requirements. The growth associated maintenance requirements are determined by fitting the model results to the experimentally determined points in the growth rate versus glucose uptake rate plot.

Next, the process 50 moves to a state 64 wherein information regarding the metabolic demands and uptake rates obtained at state 62 are combined with the genome specific stoichiometric matrix of step 8 together fully define the metabolic system using flux balance analysis (FBA). This is an approach well suited to account for genomic detail as it has been developed based on the well-known stoichiometry of metabolic reactions.

The time constants characterizing metabolic transients and/or metabolic reactions are typically very rapid, on the order of milli-seconds to seconds, compared to the time constants of cell growth on the order of hours to days. Thus, the transient mass balances can be simplified to only consider the steady state behavior. Eliminating the time derivatives obtained from dynamic mass balances around every metabolite in the metabolic system, yields the system of linear equations represented in matrix notation,

$$\mathbf{S} \cdot \mathbf{v} = \mathbf{0} \quad \text{Equation 1}$$

where \mathbf{S} refers to the stoichiometric matrix of the system, and \mathbf{v} is the flux vector. This equation simply states that over long times, the formation fluxes of a metabolite must be balanced by the degradation fluxes. Otherwise, significant amounts of the metabolite will accumulate inside the metabolic network. Applying equation 1 to our system we let \mathbf{S} now represent the genome specific stoichiometric matrix

To determine the metabolic capabilities of a defined metabolic genotype Equation 1 is solved for the metabolic fluxes and the internal metabolic reactions, \mathbf{v} , while imposing constraints on the activity of these fluxes. Typically the number of metabolic fluxes is greater than the number of mass balances (i.e., $m > n$) resulting in a plurality of feasible flux distributions that satisfy Equation 1 and any constraints placed on the fluxes of the system. This range of solutions is indicative of the flexibility in the flux distributions that can be achieved

with a given set of metabolic reactions. The solutions to Equation 1 lie in a restricted region. This subspace defines the capabilities of the *metabolic genotype* of a given organism, since the allowable solutions that satisfy Equation 1 and any constraints placed on the fluxes of the system define all the metabolic flux distributions that can be achieved with a particular set of metabolic genes.

The particular utilization of the metabolic genotype can be defined as the *metabolic phenotype* that is expressed under those particular conditions. Objectives for metabolic function can be chosen to explore the ‘best’ use of the metabolic network within a given metabolic genotype. The solution to equation 1 can be formulated as a linear programming problem, in which the flux distribution that minimizes a particular objective is found. Mathematically, this optimization can be stated as;

$$\text{Minimize } Z \quad \text{Equation 2}$$

$$\text{where } Z = \sum c_i \cdot v_i = \langle \mathbf{c} \bullet \mathbf{v} \rangle \quad \text{Equation 3}$$

where Z is the objective which is represented as a linear combination of metabolic fluxes v_i . The optimization can also be stated as the equivalent maximization problem; i.e. by changing the sign on Z .

This general representation of Z enables the formulation of a number of diverse objectives. These objectives can be design objectives for a strain, exploitation of the metabolic capabilities of a genotype, or physiologically meaningful objective functions, such as maximum cellular growth. For this application, growth is to be defined in terms of biosynthetic requirements based on literature values of biomass composition or experimentally determined values such as those obtained from state 60. Thus, we can define biomass generation as an additional reaction flux draining intermediate metabolites in the appropriate ratios and represented as an objective function Z . In addition to draining intermediate metabolites this reaction flux can be formed to utilize energy molecules such as ATP, NADH and NADPH so as to incorporate any maintenance requirement that must be met. This new reaction flux then becomes another constraint/balance equation that the system must satisfy as the objective function. It is analogous to adding an addition column to the stoichiometric matrix of Equation 1 to represent such a flux to describe the production demands placed on the metabolic system.

Setting this new flux as the objective function and asking the system to maximize the value of this flux for a given set of constraints on all the other fluxes is then a method to simulate the growth of the organism.

Using linear programming, additional constraints can be placed on the value of any of the fluxes in the metabolic network.

$$\beta_j \leq v_j \leq \alpha_j$$

Equation 4

These constraints could be representative of a maximum allowable flux through a given reaction, possibly resulting from a limited amount of an enzyme present in which case the value for α_j would take on a finite value. These constraints could also be used to include the knowledge of the minimum flux through a certain metabolic reaction in which case the value for β_j would take on a finite value. Additionally, if one chooses to leave certain reversible reactions or transport fluxes to operate in a forward and reverse manner the flux may remain unconstrained by setting β_j to negative infinity and α_j to positive infinity. If reactions proceed only in the forward reaction β_j is set to zero while α_j is set to positive infinity. As an example, to simulate the event of a genetic deletion the flux through all of the corresponding metabolic reactions related to the gene in question are reduced to zero by setting β_j and α_j to be zero in Equation 4. Based on the *in vivo* environment where the bacteria lives one can determine the metabolic resources available to the cell for biosynthesis of essentially molecules for biomass. Allowing the corresponding transport fluxes to be active provides the *in silico* bacteria with inputs and outputs for substrates and by-products produced by the metabolic network. Therefore as an example, if one wished to simulate the absence of a particular growth substrate one simply constrains the corresponding transport fluxes allowing the metabolite to enter the cell to be zero by allowing β_j and α_j to be zero in Equation 4. On the other hand if a substrate is only allowed to enter or exit the cell via transport mechanisms, the corresponding fluxes can be properly constrained to reflect this scenario.

Together the linear programming representation of the genome-specific stoichiometric matrix as in Equation 1 along with any general constraints placed on the fluxes in the system, and any of the possible objective functions completes the formulation of the *in silico* bacterial

strain. The *in silico* strain can then be used to study theoretical metabolic capabilities by simulating any number of conditions and generating flux distributions through the use of linear programming. The process 50 of formulating the *in silico* strain and simulating its behavior using linear programming techniques terminates at an end state 66.

Thus, by adding or removing constraints on various fluxes in the network it is possible to (1) simulate a genetic deletion event and (2) simulate or accurately provide the network with the metabolic resources present in its *in vivo* environment. Using flux balance analysis it is possible to determine the affects of the removal or addition of particular genes and their associated reactions to the composition of the metabolic genotype on the range of possible metabolic phenotypes. If the removal/deletion does not allow the metabolic network to produce necessary precursors for growth, and the cell can not obtain these precursors from its environment, the deletion(s) has the potential as an antimicrobial drug target. Thus by adjusting the constraints and defining the objective function we can explore the capabilities of the metabolic genotype using linear programming to optimize the flux distribution through the metabolic network. This creates what we will refer to as an *in silico* bacterial strain capable of being studied and manipulated to analyze, interpret, and predict the genotype-phenotype relationship. It can be applied to assess the affects of incremental changes in the genotype or changing environmental conditions, and provide a tool for computer aided experimental design. It should be realized that other types of organisms can similarly be represented *in silico* and still be within the scope of the invention.

The construction of a genome specific stoichiometric matrix and *in silico* microbial strains can also be applied to the area of signal transduction. The components of signaling networks can be identified within a genome and used to construct a content matrix that can be further analyzed using various techniques to be determined in the future.

A. Example 1: E. coli metabolic genotype and in silico model

Using the methods disclosed in Figures 1 and 2, an *in silico* strain of *Escherichia coli* K-12 has been constructed and represents the first such strain of a bacteria largely generated from annotated sequence data and from biochemical information. The genetic sequence and open reading frame identifications and assignments are readily available from a number of on-line

locations (ex: www.tigr.org). For this example we obtained the annotated sequence from the following website for the *E. coli* Genome Project at the University of Wisconsin (<http://www.genetics.wisc.edu/>). Details regarding the actual sequencing and annotation of the sequence can be found at that site. From the genome annotation data the subset of genes involved in cellular metabolism was determined as described above in Figure 1, state 20, comprising the metabolic genotype of the particular strain of *E. coli*.

Through detailed analysis of the published biochemical literature on *E. coli* we determined (1) all of the reactions associated with the genes in the metabolic genotype and (2) any additional reactions known to occur from biochemical data which were not represented by the genes in the metabolic genotype. This provided all of the necessary information to construct the genome specific stoichiometric matrix for *E. coli* K-12.

Briefly, the *E. coli* K-12 bacterial metabolic genotype and more specifically the genome specific stoichiometric matrix contains 731 metabolic processes that influence 436 metabolites (dimensions of the genome specific stoichiometric matrix are 436 x 731). There are 80 reactions present in the genome specific stoichiometric matrix that do not have a genetic assignment in the annotated genome, but are known to be present from biochemical data. The genes contained within this metabolic genotype are shown in Table 1 along with the corresponding reactions they carry out.

Because *E. coli* is arguably the best studied organism, it was possible to determine the uptake rates and maintenance requirements (state 62 of Figure 2) by reference to the published literature. This *in silico* strain accounts for the metabolic capabilities of *E. coli*. It includes membrane transport processes, the central catabolic pathways, utilization of alternative carbon sources and the biosynthetic pathways that generate all the components of the biomass. In the case of *E. coli* K-12, we can call upon the wealth of data on overall metabolic behavior and detailed biochemical information about the *in vivo* genotype to which we can compare the behavior of the *in silico* strain. One utility of FBA is the ability to learn about the physiology of the particular organism and explore its metabolic capabilities without any specific biochemical data. This ability is important considering possible future scenarios in which the only data that we may have for a newly discovered bacterium (perhaps pathogenic) could be its genome sequence.

B. Example 2: in silico deletion analysis for *E. coli* to find antimicrobial targets

Using the *in silico* strain constructed in Example 1, the effect of individual deletions of all the enzymes in central metabolism can be examined *in silico*. For the analysis to determine sensitive linkages in the metabolic network of *E. coli*, the objective function utilized is the maximization of the biomass yield. This is defined as a flux draining the necessary biosynthetic precursors in the appropriate ratios. This flux is defined as the biomass composition, which can be determined from the literature. See Neidhardt et. al., *Escherichia coli and Salmonella: Cellular and Molecular Biology*, Second Edition, ASM Press, Washington D.C., 1996. Thus, the objective function is the maximization of a single flux, this biosynthetic flux.

Constraints are placed on the network to account for the availability of substrates for the growth of *E. coli*. In the initial deletion analysis, growth was simulated in an aerobic glucose minimal media culture. Therefore, the constraints are set to allow for the components included in the media to be taken up. The specific uptake rate can be included if the value is known, otherwise, an unlimited supply can be provided. The uptake rate of glucose and oxygen have been determined for *E. coli* (Neidhardt et. al., *Escherichia coli and Salmonella: Cellular and Molecular Biology*, Second Edition, ASM Press, Washington D.C., 1996. Therefore, these values are included in the analysis. The uptake rate for phosphate, sulfur, and nitrogen source is not precisely known, so constraints on the fluxes for the uptake of these important substrates is not included, and the metabolic network is allowed to take up any required amount of these substrates.

The results showed that a high degree of redundancy exists in central intermediary metabolism during growth in glucose minimal media, which is related to the interconnectivity of the metabolic reactions. Only a few metabolic functions were found to be essential such that their loss removes the capability of cellular growth on glucose. For growth on glucose, the essential gene products are involved in the 3-carbon stage of glycolysis, three reactions of the TCA cycle, and several points within the PPP. Deletions in the 6-carbon stage of glycolysis result in a reduced ability to support growth due to the diversion of additional flux through the PPP.

The results from the gene deletion study can be directly compared with growth data from mutants. The growth characteristics of a series of *E. coli* mutants on several different carbon sources were examined (80 cases were determined from the literature), and compared to the *in silico* deletion results (Table 2). The majority (73 of 80 cases or 91%) of the mutant experimental observations are consistent with the predictions of the *in silico* study. The results from the *in silico* gene deletion analysis are thus consistent with experimental observations.

C. Example 3: Prediction of genome scale shifts in gene expression

Flux based analysis can be used to predict metabolic phenotypes under different growth conditions, such as substrate and oxygen availability. The relation between the flux value and the gene expression levels is non-linear, resulting in bifurcations and multiple steady states. However, FBA can give qualitative (on/off) information as well as the relative importance of gene products under a given condition. Based on the magnitude of the metabolic fluxes, qualitative assessment of gene expression can be inferred.

Figure 3a shows the five phases of distinct metabolic behavior of *E. Coli* in response to varying oxygen availability, going from completely anaerobic (phase I) to completely aerobic (phase V). Figures 3b and 3c display lists of the genes that are predicted to be induced or repressed upon the shift from aerobic growth (phase V) to nearly complete anaerobic growth (phase II). The numerical values shown in Figures 3b and 3c are the fold change in the magnitude of the fluxes calculated for each of the listed enzymes.

For this example, the objective of maximization of biomass yield is utilized (as described above). The constraints on the system are also set accordingly (as described above). However, in this example, a change in the availability of a key substrate is leading to changes in the metabolic behavior. The change in the parameter is reflected as a change in the uptake flux. Therefore, the maximal allowable oxygen uptake rate is changed to generate this data. The figure demonstrates how several fluxes in the metabolic network will change as the oxygen uptake flux is continuously decreased. Therefore, the constraints on the fluxes is identical to what is described in the previous section, however, the oxygen uptake rate is set to coincide with the point in the diagram.

Corresponding experimental data sets are now becoming available. Using high-density oligonucleotide arrays the expression levels of nearly every gene in *Saccharomyces cerevisiae* can now be analyzed under various growth conditions. From these studies it was shown that nearly 90% of all yeast mRNAs are present in growth on rich and minimal media, while a large number of mRNAs were shown to be differentially expressed under these two conditions. Another recent article shows how the metabolic and genetic control of gene expression can be studied on a genomic scale using DNA microarray technology (*Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale*, Science, Vol. 278, October 24, 1997. The temporal changes in genetic expression profiles that occur during the diauxic shift in *S. cerevisiae* were observed for every known expressed sequence tag (EST) in this genome. As shown above, FBA can be used to qualitatively simulate shifts in metabolic genotype expression patterns due to alterations in growth environments. Thus, FBA can serve to complement current studies in metabolic gene expression, by providing a fundamental approach to analyze, interpret, and predict the data from such experiments.

D. Example 4: Design of defined media

An important economic consideration in large-scale bioprocesses is optimal medium formulation. FBA can be used to design such media. Following the approach defined above, a flux-balance model for the first completely sequenced free living organism, *Haemophilus influenzae*, has been generated. One application of this model is to predict a minimal defined media. It was found that *H. influenzae* can grow on the minimal defined medium as determined from the ORF assignments and predicted using FBA. Simulated bacterial growth was predicted using the following defined media: fructose, arginine, cysteine, glutamate, putrescine, spermidine, thiamin, NAD, tetrapyrrole, pantothenate, ammonia, phosphate. This predicted minimal medium was compared to the previously published defined media and was found to differ in only one compound, inosine. It is known that inosine is not required for growth, however it does serve to enhance growth. Again the *in silico* results obtained were consistent with published *in vivo* research. These results provide confidence in the use of this type of approach for the design of defined media for organisms in which there currently does not exist a defined media.

While particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art that these embodiments are exemplary rather than limiting, and the true scope of the invention is defined by the claims that follow.

Table 1

The genes included in the *E. coli* metabolic genotype along with corresponding enzymes and reactions that comprise the genome specific stoichiometric matrix. The final column indicates the presence/absence of the gene (as the number of copies) in the *E. coli* genome. Thus the presence of a gene in the *E. coli* genome indicates that the gene is part of the metabolic genotype. Reactions/Genes not present in the genome are those gathered at state 56 in Figure 2 and together with the reactions of the genes in the metabolic genotype form the columns of the genome specific stoichiometric matrix.

Enzyme	Gene	Reaction	<i>E. coli</i> Genome
Glucokinase	<i>glk</i>	GLC + ATP \rightarrow G6P + ADP	1
Glucokinase	<i>glik</i>	bDGLC + ATP \rightarrow bDG6P + ADP	1
Phosphoglucose isomerase	<i>pgi</i>	G6P \leftrightarrow F6P	1
Phosphoglucose isomerase	<i>pgi</i>	bDG6P \leftrightarrow G6P	1
Phosphoglucose isomerase	<i>pgi</i>	bDG6P \leftrightarrow F6P	1
Aldose 1-epimerase	<i>galM</i>	bDGLC \leftrightarrow GLC	1
Glucose-1-phosphatase	<i>agp</i>	G1P \rightarrow GLC + PI	1
Phosphofructokinase B	<i>pfkA</i>	F6P + ATP \rightarrow FDP + ADP	1
Phosphofructokinase B	<i>pfkB</i>	F6P + ATP \rightarrow FDP + ADP	1
Fructose-1,6-bisphosphatase	<i>fbp</i>	FDP \rightarrow F6P + PI	1
Fructose-1,6-bisphosphatate aldolase	<i>fba</i>	FDP \leftrightarrow T3P1 + T3P2	2
Triosephosphate Isomerase	<i>tpiA</i>	T3P1 \leftrightarrow T3P2	1
Methylglyoxal synthase	<i>mgsA</i>	T3P2 \rightarrow MTHGXL + PI	0
Glyceraldehyde-3-phosphate dehydrogenase-A complex	<i>gapA</i>	T3P1 + PI + NAD \leftrightarrow NADH + 13PDG	1
Glyceraldehyde-3-phosphate dehydrogenase-C complex	<i>gapC/C2</i>	T3P1 + PI + NAD \leftrightarrow NADH + 13PDG	2
Phosphoglycerate kinase	<i>pgk</i>	13PDG + ADP \leftrightarrow 3PG + ATP	1
Phosphoglycerate mutase 1	<i>gpmA</i>	3PG \leftrightarrow 2PG	1
Phosphoglycerate mutase 2	<i>gpmB</i>	3PG \leftrightarrow 2PG	1
Enolase	<i>eno</i>	2PG \leftrightarrow PEP	1
Phosphoenolpyruvate synthase	<i>ppnA</i>	PYR + ATP \rightarrow PEP + AMP + PI	1
Pyruvate Kinase II	<i>pykA</i>	PEP + ADP \rightarrow PYR + ATP	1
Pyruvate Kinase I	<i>pykF</i>	PEP + ADP \rightarrow PYR + ATP	1
Pyruvate dehydrogenase	<i>lpdA, aceEF</i>	PYR + COA + NAD \rightarrow NADH + CO2 + ACCOA	3
Glucose-1-phosphate adenylyltransferase	<i>ggC</i>	ATP + GIP \rightarrow ADPGLC + PI	1
Glycogen synthase	<i>glgA</i>	ADPGLC \rightarrow ADP + GLYCOGEN	1

Glycogen phosphorylase	<i>glgP</i>	GLYCOGEN + PI \rightarrow G1P
Maltodextrin phosphorylase	<i>malP</i>	GLYCOGEN + PI \rightarrow G1P
Glucose 6-phosphate-1-dehydrogenase	<i>zwf</i>	G6P + NADP \leftrightarrow D6PGL + NADPH
6-Phosphogluconolactonase	<i>pgl</i>	D6PGL \rightarrow D6PGC
6-Phosphogluconate dehydrogenase (decarboxylating)	<i>gnd</i>	D6PGC + NADP \rightarrow NADPH + CO2 + RL5P
Ribose-5-phosphate isomerase A	<i>rpiA</i>	RL5P \leftrightarrow R5P
Ribose-5-phosphate isomerase B	<i>rpiB</i>	RL5P \leftrightarrow R5P
Ribulose phosphate 3-epimerase	<i>rpe</i>	RL5P \leftrightarrow X5P
Transketolase I	<i>tktA</i>	R5P + X5P \leftrightarrow T3P1 + S7P
Transketolase II	<i>tktB</i>	R5P + X5P \leftrightarrow T3P1 + S7P
Transketolase I	<i>tktA</i>	X5P + E4P \leftrightarrow F6P + T3P1
Transketolase II	<i>tktB</i>	X5P + E4P \leftrightarrow F6P + T3P1
Transaldolase B	<i>talB</i>	T3P1 + S7P \leftrightarrow E4P + F6P
Phosphogluconate aldolase	<i>edd</i>	D6PGC \rightarrow 2KD6PG
2-Keto-3-deoxy-6-phosphogluconate aldolase	<i>eda</i>	2KD6PG \rightarrow T3P1 + PYR
Citrate synthase	<i>gltA</i>	ACCOA + OA \rightarrow COA + CIT
Aconitase A	<i>acnA</i>	CIT \leftrightarrow ICIT
Aconitase B	<i>acnB</i>	CIT \leftrightarrow ICIT
Isocitrate dehydrogenase	<i>icdA</i>	ICIT + NADP \leftrightarrow CO2 + NADPH + AKG
2-Ketoglutarate dehydrogenase	<i>sucAB, lpdA</i>	AKG + NAD + COA \rightarrow CO2 + NADH + SUCCOA
Succinyl-CoA synthetase	<i>sucCD</i>	SUCCOA + ADP + PI \leftrightarrow ATP + COA + SUCC
Succinate dehydrogenase	<i>sdhABCD</i>	SUCC + FAD \rightarrow FADH + FUM
Fumurate reductase	<i>frdABCD</i>	FUM + FADH \rightarrow SUCC + FAD
Fumarase A	<i>fumA</i>	FUM \leftrightarrow MAL
Fumarase B	<i>fumB</i>	FUM \leftrightarrow MAL
Fumarase C	<i>fumC</i>	FUM \leftrightarrow MAL
Malate dehydrogenase	<i>mdh</i>	MAL + NAD \leftrightarrow NADH + OA
D-Lactate dehydrogenase 1	<i>dlal</i>	PYR + NADH \leftrightarrow NAD + LAC
D-Lactate dehydrogenase 2	<i>ldhA</i>	PYR + NADH \leftrightarrow NAD + LAC
Acetaldehyde dehydrogenase	<i>adhE</i>	ACCOA +2 NADH \leftrightarrow ETH +2 NAD + COA
Pyruvate formate lyase 1	<i>pflAB</i>	PYR + COA \rightarrow ACCOA + FOR
Pyruvate formate lyase 2	<i>pflCD</i>	PYR + COA \rightarrow ACCOA + FOR
Formate hydrogen lyase	<i>fhlF, hycBEFG</i>	FOR \rightarrow CO2
Phosphotransacetylase	<i>pta</i>	ACCOA + PI \leftrightarrow ACTP + COA
Acetate kinase A	<i>ackA</i>	ACTP + ADP \leftrightarrow ATP + AC
GAR transformylase T	<i>purT</i>	ACTP + ADP \leftrightarrow ATP + AC
Acetyl-CoA synthetase	<i>acs</i>	ATP + AC + COA \rightarrow AMP + PPI + ACCOA
Phosphoenolpyruvate carboxykinase	<i>pckA</i>	OA + ATP \rightarrow PEP + CO2 + ADP

Phosphoenolpyruvate carboxylase	1	
Malic enzyme (NADP)	0	
Malic enzyme (NAD)	1	
Isocitrate lyase	1	
Malate synthase A	1	
Malate synthase G	1	
Inorganic pyrophosphatase	1	
NADH dehydrogenase II	1	
NADH dehydrogenase I	1	
Formate dehydrogenase-N	3	
Formate dehydrogenase-O	3	
Formate dehydrogenase	1	
Pyruvate oxidase	1	
Glycerol-3-phosphate dehydrogenase (aerobic)	1	
Glycerol-3-phosphate dehydrogenase (anaerobic)	1	
Cytochrome oxidase bo3	3	
Cytochrome oxidase bd	6	
Succinate dehydrogenase complex	6	
Thioredoxin reductase	4	
Pyridine nucleotide transhydrogenase	1	
Pyridine nucleotide transhydrogenase	2	
Hydrogenase 1	2	
Hydrogenase 2	2	
Hydrogenase 3	4	
F0F1-ATPase	9	
Alpha-galactosidase (melibiose)	1	
Galactokinase	1	
Galactose-1-phosphate uridylyltransferase	1	
UDP-glucose 4-epimerase	1	
UDP-glucose-1-phosphate uridylyltransferase	1	
Phosphoglucomutase	1	
Periplasmic beta-glucosidase precursor	1	
Beta-galactosidase (LACTase)	1	
trehalose-6-phosphate hydrolase	1	
Beta-fructofuranosidase	0	
1-Phosphofructokinase (Fructose 1-phosphate kinase)	1	
Xylose isomerase	1	
Phosphomannomutase	1	
ppc	PEP + CO2 \rightarrow OA + PI	
mcE	MAL + NADP \rightarrow CO2 + NADPH + PYR	
sfCA	MAL + NAD \rightarrow CO2 + NADH + PYR	
aceA	ICIT \rightarrow GLX + SUCC	
aceB	ACCOA + GLX \rightarrow COA + MAL	
glcB	ACCOA + GLX \rightarrow COA + MAL	
ppa	PPI \rightarrow 2 PI	
ndh	NADH + Q \rightarrow NAD + QH2	
nuoABEFGHIJ	NADH + Q \rightarrow NAD + QH2 +3.5 HEXT	
fdoGH	FOR + Q \rightarrow QH2 + CO2 +2 HEXT	
fdoHFG	FOR + Q \rightarrow QH2 + CO2 +2 HEXT	
fdhF	FOR + Q \rightarrow QH2 + CO2 +2 HEXT	
poxB	PYR + Q \rightarrow AC + CO2 + QH2	
glpD	GL3P + Q \rightarrow T3P2 + QH2	
glpABC	GL3P + Q \rightarrow T3P2 + QH2	
cvoABCD, cyc	QH2 +5 O2 \rightarrow Q +2.5 HEXT	
cycABCD, app	QH2 +5 O2 \rightarrow Q +2 HEXT	
sdhABCD	FADH + Q \leftrightarrow FAD + QH2	
trxB	OTHIO + NADPH \rightarrow NADP + RTHIO	
pntAB	NADPH + NAD \rightarrow NADP + NADH	
pntAB	NADP + NADH +2 HEXT \rightarrow NADPH + NAD	
hyABC	2 Q+2 HEXT \leftrightarrow 2 QH2 + H2	
hybAC	2 Q+2 HEXT \leftrightarrow 2 QH2 + H2	
hycFGBE	2 Q+2 HEXT \leftrightarrow 2 QH2 + H2	
atpABCDEF	ATP \leftrightarrow ADP + PI +4 HEXT	
meA	MELI \rightarrow GLC + GLAC	
gak	GLAC + ATP \rightarrow GAL1P + ADP	
gatT	GAL1P + UDPG \leftrightarrow G1P + UDPGAL	
gatE	UDPGAL \leftrightarrow UDPG	
gatU	G1P + UTP \leftrightarrow UDPG + PPI	
pgm	G1P \leftrightarrow G6P	
bgX	LCTS \rightarrow GLC + GLAC	
lacZ	LCTS \rightarrow GLC + GLAC	
treC	TRE6P \rightarrow bDG6P + GLC	
	SUC6P \rightarrow G6P + FRU	
	F1P + ATP \rightarrow FDP + ADP	
	FRU \rightarrow GLC	
	MAN6P \leftrightarrow MAN1P	

Mannose-6-phosphate isomerase	<i>manA</i>	MAN1P \leftrightarrow F6P
N-Acetylglucosamine-6-phosphate deacetylase	<i>nagA</i>	NAGP \rightarrow GA6P + AC
Glucosamine-6-phosphate deaminase	<i>nagB</i>	GA6P \rightarrow F6P + NH3
N-Acetylneuraminate lyase	<i>nanA</i>	SLA \rightarrow PYR + NAMAN
L-Fucose isomerase	<i>fucI</i>	FUC \leftrightarrow FCL
L-Fuculokinase	<i>fucK</i>	FCL + ATP \rightarrow FCL1P + ADP
L-Fuculose phosphate aldolase	<i>fucA</i>	FCL1P \leftrightarrow LACAL + T3P2
Lactaldehyde reductase	<i>fucO</i>	LACAL + NADH \leftrightarrow 12PPD + NAD
Aldehyde dehydrogenase A	<i>aldA</i>	LACAL + NAD \leftrightarrow LLAC + NADH
Aldehyde dehydrogenase B	<i>aldB</i>	LACAL + NAD \leftrightarrow LLAC + NADH
Aldehyde dehydrogenase	<i>adhC</i>	LACAL + NAD \leftrightarrow LLAC + NADH
Aldehyde dehydrogenase	<i>adhC</i>	GLAL + NADH \leftrightarrow GL + NAD
Aldehyde dehydrogenase	<i>adhE</i>	LACAL + NAD \rightarrow LLAC + NADH
Aldehyde dehydrogenase	<i>aldH</i>	LACAL + NAD \leftrightarrow LLAC + NADH
Aldehyde dehydrogenase	<i>aldH</i>	ACAL + NAD \rightarrow AC + NADH
Gluconokinase I	<i>gnlV</i>	GLCN + ATP \rightarrow D6PGC + ADP
Gluconokinase II	<i>gnlK</i>	GLCN + ATP \rightarrow D6PGC + ADP
L-Rhamnose isomerase	<i>rhaA</i>	RMN \leftrightarrow RML
Rhamnulokinase	<i>rhaB</i>	RML + ATP \rightarrow RML1P + ADP
Rhamnulose-1-phosphate aldolase	<i>rhaD</i>	RML1P \leftrightarrow LACAL + T3P2
L-Arabinose isomerase	<i>araA</i>	ARAB \leftrightarrow RBL
Arabinose-5-phosphate isomerase		RL5P \leftrightarrow A5P
L-Ribulokinase		RBL + ATP \rightarrow RL5P + ADP
Rhamnulose-1-phosphate 4-epimerase		RL5P \leftrightarrow X5P
Xylose isomerase		XYL \leftrightarrow XUL
Xylulokinase		XUL + ATP \rightarrow X5P + ADP
Ribokinase		RIB + ATP \rightarrow R5P + ADP
Mannitol-1-phosphate 5-dehydrogenase	<i>rbsK</i>	MNT6P + NAD \leftrightarrow F6P + NADH
Glucitol-6-phosphate dehydrogenase	<i>mtlD</i>	GLT6P + NAD \leftrightarrow F6P + NADH
Galactitol-1-phosphate dehydrogenase	<i>srID</i>	GLT1P + NAD \leftrightarrow TAG6P + NADH
Phosphofructokinase B	<i>gatD</i>	TAG6P + ATP \rightarrow TAG16P + ADP
1-Phosphofructokinase	<i>pfkB</i>	TAG6P + ATP \rightarrow TAG16P + ADP
Tagatose-6-phosphate kinase	<i>fruk</i>	TAG6P + ATP \rightarrow TAG16P + ADP
Tagatose-bisphosphate aldolase 2	<i>agaz</i>	TAG16P \leftrightarrow T3P2 + T3P1
Tagatose-bisphosphate aldolase 1	<i>gatY</i>	TAG16P \leftrightarrow T3P2 + T3P1
Glycerol kinase	<i>agay</i>	GL + ATP \rightarrow GL3P + ADP
Glycerol-3-phosphate-dehydrogenase-[NAD(P)+]	<i>glpK</i>	GL3P + NADP \leftrightarrow T3P2 + NADPH
Phosphopentomutase	<i>gpxA</i>	DR1P \leftrightarrow DR5P
	<i>deoB</i>	

Phosphopentomutase	1
Deoxyribose-phosphate aldolase	1
Aspartate transaminase	1
Asparagine synthetase (Glutamate dependent)	1
Aspartate-ammonia ligase	1
Glutamate dehydrogenase	1
Glutamate-ammonia ligase	1
Glutamate synthase	1
Alanine transaminase	2
Valine-pyruvate aminotransferase	0
Alanine racemase, biosynthetic	1
Alanine racemase, catabolic	1
N-Acetylglutamate synthase	1
N-Acetylglutamate kinase	1
N-Acetylglutamate phosphate reductase	1
Acetylornithine transaminase	1
Acetylornithine deacetylase	1
Ornithine carbamoyl transferase 1	2
Ornithine carbamoyl transferase 2	1
Ornithine transaminase	2
Argininosuccinate synthetase	1
Argininosuccinate lyase	1
Arginine decarboxylase, biosynthetic	1
Arginine decarboxylase, degradative	1
Agmatinase	1
Ornithine decarboxylase, biosynthetic	1
Ornithine decarboxylase, degradative	1
Adenosylmethionine decarboxylase	1
Spermidine synthase	1
Methylthioadenosine nucleosidase	0
5-Methylthioribose kinase	0
5-Methylthioribose-1-phosphate isomerase	0
E-1 (Enolase-phosphatase)	0
E-3 (Unknown)	0
Transamination (Unknown)	0
γ -Glutamyl kinase	1
Glutamate-5-semialdehyde dehydrogenase	1
<i>deoB</i>	RIP \leftrightarrow R5P
<i>deoC</i>	DR5P \rightarrow ACAL + T3P1
<i>aspC</i>	OA + GLU \leftrightarrow ASP + AKG
<i>asmB</i>	ASP + ATP + GLN \rightarrow GLU + ASN + AMP + PPI
<i>asmA</i>	ASP + ATP + NH3 \rightarrow ASN + AMP + PPI
<i>gdhA</i>	AKG + NH3 + NADPH \leftrightarrow GLU + NADP
<i>ghnA</i>	GLU + NH3 + ATP \rightarrow GLN + ADP + PI
<i>glBD</i>	AKG + GLN + NADPH \rightarrow NADP + 2 GLU
<i>alaB</i>	PYR + GLU \leftrightarrow AKG + ALA
<i>avaA</i>	OVAL + ALA \rightarrow PYR + VAL
<i>ala</i>	ALA \leftrightarrow DALA
<i>dalA</i>	ALA \rightarrow DALA
<i>dadX</i>	GLU + ACCOA \rightarrow COA + NAGLU
<i>argA</i>	NAGLU + ATP \rightarrow ADP + NAGLUYP
<i>argB</i>	NAGLUYP + NADPH \leftrightarrow NADP + PI + NAGLU
<i>argC</i>	NAGLU
<i>argD</i>	NAGLU + GLU \leftrightarrow AKG + NAARON
<i>argE</i>	NAARON \rightarrow AC + ORN
<i>carAB</i>	GLN +2 ATP + CO2 \rightarrow GLU + CAP +2 ADP + PI
<i>argF</i>	ORN + CAP \leftrightarrow CTR + PI
<i>argI</i>	ORN + CAP \leftrightarrow CTR + PI
<i>ArgGH</i>	ORN + AKG \rightarrow GLUGSAL + GLU
<i>argG</i>	CTR + ASP + ATP \rightarrow AMP + PPI + ARGSUCC
<i>argH</i>	ARGSUCC \leftrightarrow FUM + ARG
<i>speA</i>	ARG \rightarrow CO2 + AGM
<i>adi</i>	ARG \rightarrow CO2 + AGM
<i>speB</i>	AGM \rightarrow UREA + PTRC
<i>speC</i>	ORN \rightarrow PTRC + CO2
<i>speF</i>	ORN \rightarrow PTRC + CO2
<i>speD</i>	SAM \leftrightarrow DSAM + CO2
<i>speE</i>	PTRC + DSAM \rightarrow SPMD + 5MTA
	5MTA \rightarrow AD + 5MTR
	5MTR + ATP \rightarrow 5MTRP + ADP
	5MTRP \leftrightarrow 5MTR1P
	5MTR1P \rightarrow DKMPP
	DKMPP \rightarrow FOR + KMB
	KMB + GLN \rightarrow GLU + MET
	GLU + ATP \rightarrow ADP + GLUP
	GLUP + NADPH \rightarrow NADP + PI + GLUGSAL

N-Acetylornithine deacetylase	<i>argE</i>	NAGLUSAL \rightarrow GLUGSAL + AC
Pyrroline-5-carboxylate reductase	<i>proC</i>	GLUGSAL + NADPH \rightarrow PRO + NADP
Threonine dehydratase, biosynthetic	<i>ihvA</i>	THR \rightarrow NH3 + OBUT
Threonine dehydratase, catabolic	<i>tdcB</i>	THR \rightarrow NH3 + OBUT
Acetohydroxybutanoate synthase I	<i>ihvBN</i>	OBUT + PYR \rightarrow ABUT + CO2
Acetohydroxybutanoate synthase II	<i>ihvGM</i>	OBUT + PYR \rightarrow ABUT + CO2
Acetohydroxybutanoate synthase III	<i>ihvIH</i>	OBUT + PYR \rightarrow ABUT + CO2
Acetohydroxy Acid isomeroeductase	<i>ihvC</i>	ABUT + NADPH \rightarrow NADP + DHMVA
Dihydroxy acid dehydratase	<i>ihvD</i>	DHMVA \rightarrow OMVAL
Branched chain amino acid aminotransferase	<i>ihvE</i>	OMVAL + GLU \leftrightarrow AKG + ILE
Acetolactate synthase I	<i>ihvBN</i>	2 PYR \rightarrow CO2 + ACLAC
Acetolactate synthase II	<i>ihvGM</i>	2 PYR \rightarrow CO2 + ACLAC
Acetolactate synthase III	<i>ihvIH</i>	2 PYR \rightarrow CO2 + ACLAC
Acetohydroxy acid isomeroeductase	<i>ihvC</i>	ACLAC + NADPH \rightarrow NADP + DHVAL
Dihydroxy acid dehydratase	<i>ihvD</i>	DHVAL \rightarrow OIVAL
Branched chain amino acid aminotransferase	<i>ihvE</i>	OIVAL + GLU \rightarrow AKG + VAL
Valine-pyruvate aminotransferase	<i>avaA</i>	OIVAL + ALA \rightarrow PYR + VAL
Isopropylmalate synthase	<i>leuA</i>	ACCOA + OIVAL \rightarrow COA + CBHCAp
Isopropylmalate isomerase	<i>leuCD</i>	CBHCAp \leftrightarrow IPPMAL
3-Isopropylmalate dehydrogenase	<i>leuB</i>	IPPMAL + NAD \rightarrow NADH + OICAP + CO2
Branched chain amino acid aminotransferase	<i>ihvE</i>	OICAP + GLU \rightarrow AKG + LEU
Aromatic amino acid transaminase	<i>tyrB</i>	OICAP + GLU \rightarrow AKG + LEU
2-Dehydro-3-deoxyphosphoheptonate aldolase F	<i>aroF</i>	E4P + PEP \rightarrow PI + 3DDAHTP
2-Dehydro-3-deoxyphosphoheptonate aldolase G	<i>aroG</i>	E4P + PEP \rightarrow PI + 3DDAHTP
2-Dehydro-3-deoxyphosphoheptonate aldolase H	<i>aroH</i>	E4P + PEP \rightarrow PI + 3DDAHTP
3-Dehydroquinate synthase	<i>aroB</i>	3DDAHTP \rightarrow DQT + PI
3-Dehydroquinate dehydrogenase	<i>aroD</i>	DQT \leftrightarrow DHSK
Shikimate dehydrogenase	<i>aroE</i>	DHSK + NADPH \leftrightarrow SME + NADP
Shikimate kinase I	<i>aroK</i>	SME + ATP \rightarrow ADP + SME5P
Shikimate kinase II	<i>aroL</i>	SME + ATP \rightarrow ADP + SME5P
3-Phosphoshikimate-1-carboxyvinyltransferase	<i>aroA</i>	SME5P + PEP \leftrightarrow 3PSME + PI
Chorismate synthase	<i>aroC</i>	3PSME \rightarrow PI + CHOR
Chorismate mutase 1	<i>pheA</i>	CHOR \rightarrow PHEN
Prephenate dehydratase	<i>tyrB</i>	PHEN \rightarrow CO2 + PHPYR
Aromatic amino acid transaminase	<i>tyrA</i>	PHPYR + GLU \leftrightarrow AKG + PHE
Chorismate mutase 2	<i>tyrB</i>	CHOR \rightarrow PHEN
Prephenate dehydratase	<i>tyrA</i>	PHEN + NAD \rightarrow PHPYR + CO2 + NADH
Aromatic amino acid transaminase	<i>tyrB</i>	PHPYR + GLU \leftrightarrow AKG + TYR

Aspartate transaminase	<i>aspC</i>	HPHPYR + GLU \leftrightarrow AKG + TYR
Anthranilate synthase	<i>trpDE</i>	CHOR + GLN \rightarrow GLU + PYR + AN
Anthranilate synthase component II	<i>trpD</i>	AN + PRPP \rightarrow PPI + NPNAN
Phosphoribosyl anthranilate isomerase	<i>trpC</i>	NPNAN \rightarrow CPAD5P
Indoleglycerol phosphate synthase	<i>trpC</i>	CPAD5P \rightarrow CO2 + IGP
Tryptophan synthase	<i>trpAB</i>	IGP + SER \rightarrow T3P1 + TRP
Phosphoribosyl pyrophosphate synthase	<i>prsA</i>	R5P + ATP \leftrightarrow PRPP + AMP
ATP phosphoribosyltransferase	<i>hisG</i>	PRPP + ATP \rightarrow PPI + PRBATP
Phosphoribosyl-A TP pyrophosphatase	<i>hisE</i>	PRBATP \rightarrow PPI + PRBAMP
Phosphoribosyl-AMP cyclohydrolase	<i>hisA</i>	PRBAMP \rightarrow PRFP
Phosphoribosylformimino-5-amino-1-phosphoribosyl-4-imidazole c	<i>hisFH</i>	PRFP \rightarrow PRLP
Imidazoleglycerol phosphate synthase	<i>hisB</i>	PRLP + GLN \rightarrow GLU + AICAR + DIMGP
L-Histidinol phosphate aminotransferase	<i>hisC</i>	DIMGP \rightarrow IMACP
Histidinol phosphatase	<i>hisB</i>	IMACP + GLU \rightarrow AKG + HISOLP
Histidinol dehydrogenase	<i>hisD</i>	HISOLP \rightarrow PI + HISOL
3-Phosphoglycerate dehydrogenase	<i>serA</i>	HISOL + 3 NAD \rightarrow HIS + 3 NADH
Phosphoserine transaminase	<i>serC</i>	3PG + NAD \rightarrow NADH + PHP
Phosphoserine phosphatase	<i>serB</i>	PHP + GLU \rightarrow AKG + 3PSER
Glycine hydroxymethyltransferase	<i>glyA</i>	3PSER \rightarrow PI + SER
Threonine dehydrogenase	<i>tah</i>	THF + SER \rightarrow GLY + MERTHF
Amino ketobutyrate CoA ligase	<i>kbI</i>	THR + COA \rightarrow GLY + ACCOA
Sulfate adenylyltransferase	<i>cysDN</i>	THR + COA \rightarrow GLY + ACCOA
Adenylylsulfate kinase	<i>cysC</i>	SLF + ATP + GTP \rightarrow PPI + APS + GDP + PI
3'-Phospho-adenylylsulfate reductase	<i>cysH</i>	APS + ATP \rightarrow ADP + PAPS
Sulfite reductase	<i>cysU</i>	PAPS + RTHIO \rightarrow OTHIO + H2SO3 + PAP
Serine transacetylase	<i>cysE</i>	H2SO3 + 3 NADPH \leftrightarrow H2S + 3 NADP
O-Acetylserine (thiol)-lyase A	<i>cysK</i>	SER + ACCOA \leftrightarrow COA + ASER
O-Acetylserine (thiol)-lyase B	<i>cysM</i>	ASER + H2S \rightarrow AC + CYS
3' - 5' Bisphosphate nucleotidase		ASER + H2S \rightarrow AC + CYS
Aspartate kinase I		PAP \rightarrow AMP + PI
Aspartate kinase II	<i>thrA</i>	ASP + ATP \leftrightarrow ADP + BASP
Aspartate kinase III	<i>metL</i>	ASP + ATP \leftrightarrow ADP + BASP
Aspartate semialdehyde dehydrogenase	<i>lysC</i>	ASP + ATP \leftrightarrow ADP + BASP
Homoserine dehydrogenase I	<i>asd</i>	BASP + NADPH \leftrightarrow NADP + PI + ASPSA
Homoserine dehydrogenase II	<i>thrA</i>	ASPSA + NADPH \leftrightarrow NADP + HSER
Homoserine kinase	<i>metL</i>	ASPSA + NADPH \leftrightarrow NADP + HSER
Threonine synthase	<i>thrB</i>	HSER + ATP \rightarrow ADP + PHSER
	<i>thrC</i>	PHSER \rightarrow PI + THR

It is much more difficult to find out what has been done with the money.

Dihydriodicolinate synthase	<i>dapsA</i>	ASPSA + PYR \rightarrow D23PIC
Dihydriodicolinate reductase	<i>dapB</i>	D23PIC + NADPH \rightarrow NADP + PIP26DX
Tetrahydriodicolimate succinylase	<i>dapD</i>	PIP26DX + SUCCOA \rightarrow COA + NS2A6O
Succinyl diaminopimelate aminotransferase	<i>dapC</i>	NS2A6O + GLU \leftrightarrow AKG + NS26DP
Succinyl diaminopimelate desuccinylase	<i>dapE</i>	NS26DP \rightarrow SUCC + D26PIM
Diaminopimelate epimerase	<i>dapF</i>	D26PIM \leftrightarrow MDAP
Diaminopimelate decarboxylase	<i>lysA</i>	MDAP \rightarrow CO2 + LYS
Lysine decarboxylase 1	<i>cadA</i>	LYS \rightarrow CO2 + CADV
Lysine decarboxylase 2	<i>ldcC</i>	LYS \rightarrow CO2 + CADV
Homoserine transsuccinylase	<i>metaA</i>	HSER + SUCCOA \rightarrow COA + OSLHSER
O-succinylhomoserine lyase	<i>metB</i>	OSLHSER + CYS \rightarrow SUCC + LLCT
Cystathione- β -lyase	<i>metC</i>	LLCT \rightarrow HCYS + PYR + NH3
Adenosyl homocysteine (Unknown)	<i>Unknown</i>	HCYS + ADN \leftrightarrow SAH
Cobalamin-dependent methionine synthase	<i>methH</i>	HCYS + MTHF \rightarrow MET + THF
Cobalamin-independent methionine synthase	<i>metE</i>	HCYS + MTHF \rightarrow MET + THF
S-Adenosylmethionine synthetase	<i>metK</i>	MET + ATP \rightarrow PPI + PI + SAM
D-Amino acid dehydrogenase	<i>dadA</i>	DALA + FAD \rightarrow FADH + PYR + NH3
Putrescine transaminase	<i>pat</i>	PTRC + AKG \rightarrow GABAL + GLU
Amino oxidase	<i>tynA</i>	PTRC \rightarrow GABAL + NH3
Aminobutyraldehyde dehydrogenase	<i>prr</i>	GABAL + NAD \rightarrow GABA + NADH
Aldehyde dehydrogenase	<i>alldH</i>	GABAL + NAD \rightarrow GABA + NADH
Aminobutyrate aminotransaminase 1	<i>gabT</i>	GABA + AKG \rightarrow SUCCSAL + GLU
Aminobutyrate aminotransaminase 2	<i>goaG</i>	GABA + AKG \rightarrow SUCCSAL + GLU
Succinate semialdehyde dehydrogenase -NAD	<i>sad</i>	SUCCSAL + NAD \rightarrow SUCC + NADH
Succinate semialdehyde dehydrogenase -NADP	<i>gabD</i>	SUCCSAL + NADP \rightarrow SUCC + NADPH
Asparinase I	<i>ansA</i>	ASN \rightarrow ASP + NH3
Asparinase II	<i>ansB</i>	ASN \rightarrow ASP + NH3
Aspartate ammonia-lyase	<i>aspA</i>	ASP \rightarrow FUM + NH3
Tryptophanase	<i>tnaA</i>	CYS \rightarrow PYR + NH3 + H2S
L-Cysteine desulphydrase	<i>gadA</i>	CYS \rightarrow PYR + NH3 + H2S
Glutamate decarboxylase A	<i>gadB</i>	GLU \rightarrow GABA + CO2
Glutamate decarboxylase B		GLU \rightarrow GABA + CO2
Glutaminase A		GLN \rightarrow GLU + NH3
Glutaminase B		GLN \rightarrow GLU + NH3
Proline dehydrogenase	<i>putA</i>	PRO + FAD \rightarrow FADH + GLUGSAL
Pyrrrolidine-5-carboxylate dehydrogenase	<i>putA</i>	GLUGSAL + NAD \rightarrow NADH + GLU
Serine deaminase 1	<i>sdaA</i>	SER \rightarrow PYR + NH3

Tryptophanase	<i>trpA</i>	SER \Rightarrow PYR + NH3
D-Serine deaminase	<i>dsdA</i>	DSER \Rightarrow PYR + NH3
Threonine dehydrogenase	<i>tdh</i>	THR + NAD \Rightarrow 2A3O + NADH
Amino ketobutyrate ligase	<i>kbI</i>	2A3O + COA \Rightarrow ACCOA + GLY
Threonine dehydratase catabolic	<i>tdcB</i>	THR \Rightarrow OBUR + NH3
Threonine deaminase 1	<i>sdaA</i>	THR \Rightarrow OBUR + NH3
Threonine deaminase 2	<i>sdaB</i>	THR \Rightarrow OBUR + NH3
Tryptophanase	<i>trpA</i>	TRP \Leftrightarrow INDOLE + PYR + NH3
Amidophosphoribosyl transferase	<i>purF</i>	PRPP + GLN \Rightarrow PPI + GLU + PRAM
Phosphoribosylamino-glycine ligase	<i>purD</i>	PRAM + ATP + GLY \Leftrightarrow ADP + PI + GAR
Phosphoribosylglycynamide formyltransferase	<i>purN</i>	GAR + FTHF \Rightarrow THF + FGAR
GAR transformylase T	<i>purT</i>	GAR + FOR + ATP \Rightarrow ADP + PI + FGAR
Phosphoribosylformylglycynamide synthetase	<i>purL</i>	FGAR + ATP + GLN \Rightarrow GLU + ADP + PI + FGAM
Phosphoribosylformylglycynamide cyclo-ligase	<i>purM</i>	FGAM + ATP \Rightarrow ADP + PI + AIR
Phosphoribosylaminomimidazole carboxylase 1	<i>purK</i>	AIR + CO2 + ATP \Leftrightarrow NCAIR + ADP + PI
Phosphoribosylaminomimidazole carboxylase 2	<i>purE</i>	NCAIR \Leftrightarrow CAIR
5'-Phosphoribosyl-4-(N-succinocarboxamido)-5-aminoimidazole 4-lyase	<i>purC</i>	CAIR + ATP + ASP \Leftrightarrow ADP + PI + SAICAR
5'-Phosphoribosyl-4-(N-succinocarboxamido)-5-aminoimidazole 4-lyase	<i>purB</i>	SAICAR \Leftrightarrow FUM + AICAR
AICAR transformylase	<i>purH</i>	AICAR + FTHF \Leftrightarrow THF + PRFICA
IMP cyclohydrolase	<i>purF</i>	PRFICA \Leftrightarrow IMP
Adenylosuccinate synthetase	<i>purA</i>	IMP + GTP + ASP \Rightarrow GDP + PI + ASUC
Adenylosuccinate lyase	<i>purB</i>	ASUC \Leftrightarrow FUM + AMP
IMP dehydrogenase	<i>guaB</i>	IMP + NAD \Rightarrow NADH + XMP
GMP synthase	<i>guaA</i>	XMP + ATP + GLN \Rightarrow GLU + AMP + PPI + GMP
GMP reductase	<i>guaC</i>	GMP + NADPH \Rightarrow NADP + IMP + NH3
Aspartate-carbamoyltransferase	<i>pyrBI</i>	CAP + ASP \Rightarrow CAAASP + PI
Dihydroorotase	<i>pyrC</i>	CAAASP \Leftrightarrow DOROA
Dihydroorotate dehydrogenase	<i>pyrD</i>	DOROA + Q \Leftrightarrow QH2 + OROA
Orotate phosphoribosyl transferase	<i>pyrE</i>	OROA + PRPP \Leftrightarrow PPI + OMP
OMP decarboxylase	<i>pyrF</i>	OMP \Rightarrow CO2 + UMP
CTP synthetase	<i>pyrG</i>	UTP + GLN + ATP \Rightarrow CTP + ADP + PI
Adenylylate kinase	<i>atk</i>	ATP + AMP \Leftrightarrow 2 ADP
Adenylylate kinase	<i>atk</i>	GTP + AMP \Leftrightarrow ADP + GDP
Adenylylate kinase	<i>atk</i>	ITP + AMP \Leftrightarrow ADP + IDP
Adenylylate kinase	<i>atk</i>	DAMP + ATP \Leftrightarrow ADP + DADP
Guanylylate kinase	<i>gmk</i>	GMP + ATP \Leftrightarrow GDP + ADP
Deoxyguanylylate kinase	<i>gmk</i>	DGMP + ATP \Leftrightarrow DGDP + ADP
Nucleoside-diphosphate kinase	<i>ndk</i>	GDP + ATP \Leftrightarrow GTP + ADP

Nucleoside-diphosphate kinase	1	NDP + ATP \leftrightarrow UTP + ADP
Nucleoside-diphosphate kinase	1	CDP + ATP \leftrightarrow CTP + ADP
Nucleoside-diphosphate kinase	1	DGDP + ATP \leftrightarrow DGTP + ADP
Nucleoside-diphosphate kinase	1	DUDP + ATP \leftrightarrow DUTP + ADP
Nucleoside-diphosphate kinase	1	DCDP + ATP \leftrightarrow DCTP + ADP
Nucleoside-diphosphate kinase	1	DADP + ATP \leftrightarrow DATP + ADP
Nucleoside-diphosphate kinase	1	DTDP + ATP \leftrightarrow DTTP + ADP
AMP Nucleosidase	1	AMP \rightarrow AD + R5P
Adenosine deaminase	1	ADN \rightarrow INS + NH3
Deoxyadenosine deaminase	1	DA \rightarrow DIN + NH3
Adenine deaminase	1	AD \rightarrow NH3 + HYXN
Inosine kinase	1	INS + ATP \rightarrow IMP + ADP
Guanosine kinase	1	GSN + ATP \rightarrow GMP + ADP
Adenosine kinase	1	ADN + ATP \rightarrow AMP + ADP
Adenine phosphoryltransferase	1	AD + PRPP \rightarrow PPI + AMP
Xanthanine-guanine phosphoribosyltransferase	1	XAN + PRPP \rightarrow XMP + PPI
Xanthanine-guanine phosphoribosyltransferase	1	HYXN + PRPP \rightarrow PPI + IMP
Hypoxanthine phosphoribosyltransferase	1	HYXN + PRPP \rightarrow PPI + IMP
Xanthanine-guanine phosphoribosyltransferase	1	GN + PRPP \rightarrow PPI + GMP
Hypoxanthine phosphoribosyltransferase	1	GN + PRPP \rightarrow PPI + GMP
Xanthosine phosphorylase	1	DIN + PI \leftrightarrow HYXN + DRIP
Purine nucleotide phosphorylase	1	DIN + PI \leftrightarrow HYXN + DRIP
Xanthosine phosphorylase	1	DA + PI \leftrightarrow AD + DRIP
Purine nucleotide phosphorylase	1	DA + PI \leftrightarrow AD + DRIP
Xanthosine phosphorylase	1	DG + PI \leftrightarrow GN + DRIP
Purine nucleotide phosphorylase	1	DG + PI \leftrightarrow GN + DRIP
Xanthosine phosphorylase	1	HYXN + RIP \leftrightarrow INS + PI
Purine nucleotide phosphorylase	1	HYXN + RIP \leftrightarrow INS + PI
Xanthosine phosphorylase	1	AD + RIP \leftrightarrow PI + ADN
Purine nucleotide phosphorylase	1	AD + RIP \leftrightarrow PI + ADN
Xanthosine phosphorylase	1	GN + RIP \leftrightarrow PI + GSN
Purine nucleotide phosphorylase	1	GN + RIP \leftrightarrow PI + GSN
Xanthosine phosphorylase	1	XAN + R1P \leftrightarrow PI + XTSN
Purine nucleotide phosphorylase	1	XAN + RIP \leftrightarrow PI + XTSN
Uridine phosphorylase	1	URI + PI \leftrightarrow URA + RIP
Thymidine (deoxyuridine) phosphorylase	1	DU + PI \leftrightarrow URA + DRIP
Purine nucleotide phosphorylase	1	DU + PI \leftrightarrow URA + DRIP
Thymidine (deoxyuridine) phosphorylase	1	DT + PI \leftrightarrow THY + DRIP

Cytidylate kinase	<i>cmkA</i>	DCMP + ATP \leftrightarrow ADP + DCDP
Cytidylate kinase	<i>cmkA</i>	CMP + ATP \leftrightarrow ADP + CDP
Cytidylate kinase	<i>cmkB</i>	DCMP + ATP \leftrightarrow ADP + DCDP
Cytidylate kinase	<i>cmkB</i>	CMP + ATP \leftrightarrow ADP + CDP
Cytidylate kinase	<i>cmkA</i>	UMP + ATP \leftrightarrow ADP + UDP
Cytidylate kinase	<i>cmkB</i>	UMP + ATP \leftrightarrow ADP + UDP
dTTP kinase	<i>tmk</i>	DTMP + ATP \leftrightarrow ADP + DTDP
Uridylate kinase	<i>pyrH</i>	UMP + ATP \leftrightarrow UDP + ADP
Uridylate kinase	<i>pyrH</i>	DUMP + ATP \leftrightarrow DUDP + ADP
Uridylate kinase	<i>tdk</i>	DU + ATP \rightarrow DUMP + ADP
Uridylate kinase	<i>upp</i>	URA + PRPP \rightarrow UMP + PPI
Cytosine deaminase	<i>cdd</i>	CYTS \rightarrow URA + NH3
Uridine kinase	<i>udk</i>	URI + GTP \rightarrow GDP + UMP
Cytidine kinase	<i>udk</i>	CYTD + GTP \rightarrow GDP + CMP
CMP glycosylase	<i>cdd</i>	CMP \rightarrow CYTS + R5P
Cytidine deaminase	<i>cdd</i>	CYTD \rightarrow URI + NH3
Thymidine (deoxyuridine) kinase	<i>tdk</i>	DT + ATP \rightarrow ADP + DTMP
Uracil phosphoribosyltransferase	<i>dcd</i>	DCTP \rightarrow DUTP + NH3
Cytosine deaminase	<i>cd</i>	DC \rightarrow NH3 + DU
Uridine kinase	<i>ushA</i>	DUMP \rightarrow DU + PI
Cytidine kinase	<i>ushA</i>	DTMP \rightarrow DT + PI
CMP glycosylase	<i>ushA</i>	DAMP \rightarrow DA + PI
Cytidine deaminase	<i>ushA</i>	DGMP \rightarrow DG + PI
Thymidine (deoxyuridine) kinase	<i>ushA</i>	DCMP \rightarrow DC + PI
dCTP deaminase	<i>ushA</i>	CMP \rightarrow CYTD + PI
Cytidine deaminase	<i>ushA</i>	AMP \rightarrow PI + ADN
5'-Nucleotidase	<i>ushA</i>	GMP \rightarrow PI + GSN
5'-Nucleotidase	<i>ushA</i>	IMP \rightarrow PI + INS
5'-Nucleotidase	<i>ushA</i>	XMP \rightarrow PI + XTSN
5'-Nucleotidase	<i>ushA</i>	UMP \rightarrow PI + URI
5'-Nucleotidase	<i>nrnAB</i>	ADP + RTHIO \rightarrow DADP + OTHIO
5'-Nucleotidase	<i>nrnAB</i>	GDP + RTHIO \rightarrow DGDP + OTHIO
5'-Nucleotidase	<i>nrnD</i>	ATP + RTHIO \rightarrow DATP + OTHIO
5'-Nucleotidase	<i>nrnD</i>	GTP + RTHIO \rightarrow DGTP + OTHIO
5'-Nucleotidase	<i>nrnAB</i>	CDP + RTHIO \rightarrow DCDP + OTHIO
5'-Nucleotidase	<i>nrnEF</i>	CDP + RTHIO \rightarrow DCDP + OTHIO
5'-Nucleotidase	<i>nrnAB</i>	UDP + RTHIO \rightarrow DUDP + OTHIO
5'-Nucleotidase	<i>nrnD</i>	CTP + RTHIO \rightarrow DCTP + OTHIO

Ribonucleoside-triphosphate reductase	<i>nrpD</i>	UTP + RTHIO \rightarrow OTHIO + DUTP	1
dUTP pyrophosphatase	<i>dut</i>	DUTP \rightarrow PP1 + DUMP	1
Thymidilate synthetase	<i>thyA</i>	DUMP + METTHF \rightarrow DHF + DTMP	1
Nucleoside triphosphatase	<i>mutT</i>	GTP \rightarrow GSN + 3 PI	1
Nucleoside triphosphatase	<i>mutT</i>	DGTP \rightarrow DG + 3 PI	1
Deoxyguanosine-triphosphate triphosphohydrolase	<i>dgt</i>	DGTP \rightarrow DG + 3 PI	1
Deoxyguanosine-triphosphate triphosphohydrolase	<i>dgt</i>	GTP \rightarrow GSN + 3 PI	1
Glycine cleavage system (Multi-component system)	<i>gcvHTP, lpdA</i>	GLY + THF + NAD \rightarrow METTHF + NADH + CO2 + NH3	4
Formyl tetrahydrofolate deformylase	<i>purU</i>	FTHF \rightarrow FOR + THF	1
Methylene tetrahydrofolate reductase	<i>metF</i>	METTHF + NADH \rightarrow NAD + MTHF	1
Methylene THF dehydrogenase	<i>folD</i>	METTHF + NADP \rightarrow METHF + NADPH	1
Methylene tetrahydrofolate cyclohydrolase	<i>folD</i>	METHF \leftrightarrow FTHF	1
Acetyl-CoA carboxyltransferase	<i>accABD</i>	ACCOA + ATP + CO2 \leftrightarrow MALCOA + ADP + PI	3
Malonyl-CoA-ACP transacylase	<i>fabD</i>	MALCOA + ACP \leftrightarrow MALACP + COA	1
Malonyl-ACP decarboxylase	<i>fabB</i>	MALACP \rightarrow ACACCP + CO2	1
Acetyl-CoA-ACP transacylase	<i>fabH</i>	ACACCP + COA \leftrightarrow ACCOA + ACP	1
Acyltransferase	<i>pls</i>	GL3P +0.035 C140ACP +0.102 C141ACP +0.717 C160AC 0	0
CDP-Diacylglycerol pyrophosphatase	<i>cdsA</i>	PA + CTP \leftrightarrow CDPDG + PPI	1
CDP-Diacylglycerol pyrophosphatase	<i>cdh</i>	CDPDG \rightarrow CMP + PA	1
Phosphatidylserine synthase	<i>pssA</i>	CDPDG + SER \leftrightarrow CMP + PS	1
Phosphatidylserine decarboxylase	<i>psd</i>	PS \rightarrow PE + CO2	1
Phosphatidylglycerol phosphate synthase	<i>pgsA</i>	CDPDG + GL3P \leftrightarrow CMP + PGP	1
Phosphatidylglycerol phosphate phosphatase A	<i>pgpA</i>	PGP \rightarrow PI + PG	0
Phosphatidylglycerol phosphate phosphatase B	<i>pgpB</i>	2 PG \leftrightarrow CL + GL	1
Cardiolipin synthase	<i>cls</i>	2 ACCOA \leftrightarrow COA + AACCOA	1
Acetyl-CoA C-acetyltransferase	<i>atob</i>	T3P1 + PYR + 2 NADPH + ATP \rightarrow IPPP + ADP + 2 NADP + 0	0
Isoprenyl-pyrophosphate synthesis pathway	<i>ispA</i>	IPPP \rightarrow DMPP	0
Isoprenyl-pyrophosphate isomerase	<i>ispA</i>	DMPP + IPPP \rightarrow GPP + PPI	1
Farnesyl pyrophosphate synthetase	<i>ispA</i>	GPP + IPPP \rightarrow FPP + PPI	1
Geranyltransferase	<i>ispB</i>	5 IPPP + FPP \rightarrow OPP + 5 PPI	1
Octoprenyl pyrophosphate synthase (5 reactions)		8 IPPP + FPP \rightarrow UDPP + 8 PPI	0
Undecaprenyl pyrophosphate synthase (8 reactions)		CHOR \rightarrow 4HBZ + PYR	1
Chorismate pyruvate-lyase	<i>ubiC</i>	4HBZ + OPP \rightarrow O4HBZ + PPI	1
Hydroxybenzoate octaprenyltransferase	<i>ubiA</i>	O4HBZ \rightarrow CO2 + 2OPPP	1
Octaprenyl-hydroxybenzoate decarboxylase	<i>ubiD, ubiX</i>	2OPPP + O2 \rightarrow 2O6H	1
2-Octaprenylphenol hydroxylase	<i>ubiB</i>	2O6H + SAM \rightarrow 2OPMP + SAH	0
Methylation reaction		2OPMP + O2 \rightarrow 2OPMB	1
2-Octaprenyl-6-methoxyphenol hydroxylase	<i>ubiH</i>		

2-Octaprenyl-6-methoxy-1,4-benzoquinone methylase	<i>ubiE</i>	2OPMB + SAM \rightarrow 2OPMMB + SAH	0
2-Octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone hydroxylase	<i>ubiF</i>	2OPMMB + O2 \rightarrow 2OMHMB	0
3-Dimethylubiquinone 3-methyltransferase	<i>ubiG</i>	2OMHMB + SAM \rightarrow QH2 + SAH	1
Isochorismate synthase 1	<i>menF</i>	CHOR \rightarrow ICHOR	1
α -Ketoglutarate decarboxylase	<i>menD</i>	AKG + TPP \rightarrow SSALTPP + CO2	1
SHCHC synthase	<i>menD</i>	ICHOR + SSALTPP \rightarrow PYR + TPP + SHCHC	1
O-Succinylbenzoate-CoA synthase	<i>menC</i>	SHCHC \rightarrow OSB	1
O-Succinylbenzoic acid-CoA ligase	<i>menE</i>	OSB + ATP + COA \rightarrow OSBCOA + AMP + PPI	1
Naphthoate synthase	<i>menB</i>	OSBCOA \rightarrow DHNA + COA	1
1,4-Dihydroxy-2-naphthoate octaprenyltransferase	<i>menA</i>	DHNA + OPP \rightarrow DMK + PPI + CO2	1
S-Adenosylmethionine-2-DMK methyltransferase	<i>menG</i>	DMK + SAM \rightarrow MK + SAH	1
Isochorismate synthase 2	<i>entC</i>	CHOR \rightarrow ICHOR	1
Isochorismatase	<i>entB</i>	ICHOR \rightarrow 23DHDB + PYR	1
2,3-Dihydro-2,3-dihydroxybenzoate dehydrogenase	<i>entA</i>	23DHDB + NAD \rightarrow 23DHDB + NADH	1
ATP-dependent activation of 2,3-dihydroxybenzoate	<i>entE</i>	23DHDB + ATP \rightarrow 23DHBA + PPI	1
ATP-dependent serine activating enzyme	<i>entF</i>	SER + ATP \leftrightarrow SERA + PPI	1
Enterochelin synthetase	<i>entD</i>	3 SERA +3 23DHBA \rightarrow ENTER +6 AMP	1
GTP cyclohydrolase II	<i>ribA</i>	GTP \rightarrow D6RP5P + FOR + PPI	1
Pyrimidine deaminase	<i>ribD</i>	D6RP5P \rightarrow A6RP5P + NH3	1
Pyrimidine reductase	<i>ribD</i>	A6RP5P + NADPH \rightarrow A6RP5P2 + NADP	1
Pyrimidine phosphatase	<i>ribB</i>	A6RP5P2 \rightarrow A6RP + PI	0
3,4 Dihydroxy-2-butanone-4-phosphate synthase	<i>ribE</i>	RL5P \rightarrow DB4P + FOR	1
6,7-Dimethyl-8-ribityllumazine synthase	<i>ribH</i>	DB4P + A6RP \rightarrow D8RL + PI	1
Riboflavin synthase	<i>ribF</i>	2 D8RL \rightarrow RIBFLV + A6RP	1
Riboflavin kinase	<i>ribF</i>	RIBFLV + ATP \rightarrow FMN + ADP	1
FAD synthetase	<i>folE</i>	FMN + ATP \rightarrow FAD + PPI	1
GTP cyclohydrolase I	<i>folE</i>	GTP \rightarrow FOR + AHTD	1
Dihydronoepterin triphosphate pyrophosphorylase	<i>ntpA</i>	AHTD \rightarrow PPI + DHPP	1
Nucleoside triphosphatase	<i>mutT</i>	AHTD \rightarrow DHP +3 PI	1
Dihydronoepterin monophosphate dephosphorylase	<i>folB</i>	DHP \rightarrow DHP + PI	0
Dihydronoepterin aldolase	<i>folK</i>	DHP \rightarrow AHHMP + GLAL	1
6-Hydroxymethyl-7,8 dihydopterin pyrophosphokinase	<i>folK</i>	AHHMP + ATP \rightarrow AMP + AHHMD	1
Aminodeoxychorismate synthase	<i>pabAB</i>	CHOR + GLN \rightarrow ADCHOR + GLU	2
Aminodeoxychorismate lyase	<i>pabC</i>	ADCHOR \rightarrow PYR + PABA	1
Dihydropteroate synthase	<i>folP</i>	PABA + AHHMD \rightarrow PPI + DHPP	1
Dihydrofolate synthetase	<i>folC</i>	DHPP + ATP + GLU \rightarrow ADP + PI + DHF	1
Dihydrofolate reductase	<i>folA</i>	DHF + NADPH \rightarrow NADP + THF	1
Ketopentoate hydroxymethyl transferase	<i>panB</i>	OIVAL + METTHF \rightarrow AKP + THF	1

Uroporphyrinogen decarboxylase	<i>hemE</i>	UPRG -> 4 CO ₂ + CPP
Coproporphyrinogen oxidase, aerobic	<i>hemF</i>	O ₂ + CPP -> 2 CO ₂ + PPHG
Protoporphyrinogen oxidase	<i>hemG</i>	O ₂ + PPHG -> PPIX
Ferrochelatase	<i>hemH</i>	PPIX -> PTH
Heme O synthase	<i>cyoE</i>	PTH + FPP -> HO + PPI
8-Amino-7-oxononanoate synthase	<i>bioF</i>	ALA + CHCOA -> CO ₂ + COA + AONA
Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	<i>bioA</i>	SAM + AONA -> SAMOB + DANNA
Dethiobiotin synthase	<i>bioD</i>	CO ₂ + DANNA + ATP -> DTB + PI + ADP
Biotin synthase	<i>bioB</i>	DTB + CYS -> BT
Glutamate-cysteine ligase	<i>gshA</i>	CYS + GLU + ATP -> GC + PI + ADP
Glutathione synthase	<i>gshB</i>	GLY + GC + ATP -> RGT + PI + ADP
Glutathione reductase	<i>gor</i>	NADPH + OGT -> NADP + RGT
thiC protein	<i>thiC</i>	AIR -> AHM
HMP kinase	<i>thiN</i>	AHM + ATP -> AHMPP + ADP
HMP-phosphate kinase	<i>thiD</i>	AHMPP + ATP -> AHMPP + ADP
Hypothetical		T3P1 + PYR -> DTP
thiG protein	<i>thiG</i>	DTP + TYR + CYS -> THZ + HBA + CO ₂
thiE protein	<i>thiE</i>	DTP + TYR + CYS -> THZ + HBA + CO ₂
thiF protein	<i>thiF</i>	DTP + TYR + CYS -> THZ + HBA + CO ₂
thiH protein	<i>thiH</i>	DTP + TYR + CYS -> THZ + HBA + CO ₂
THZ kinase	<i>thiM</i>	THZ + ATP -> THZP + ADP
Thiamin phosphate synthase	<i>thiB</i>	THZP + AHMPP -> THMP + PPI
Thiamin kinase	<i>thiK</i>	THMP + ADP -> THIAMIN + ATP
Thiamin phosphate kinase	<i>thiL</i>	THMP + ATP -> TPP + ADP
Erythrose 4-phosphate dehydrogenase	<i>epd</i>	E4P + NAD -> ER4P + NADH
Erythronate-4-phosphate dehydrogenase	<i>paxB</i>	ER4P + NAD -> OHB + NADH
Hypothetical transaminase/phosphoserine transaminase	<i>serC</i>	OHB + GLU -> PHT + AKG
Pyridoxal-phosphate biosynthetic proteins pdxJ-pdxA	<i>pdxAJ</i>	PHT + DXSP -> P5P + CO ₂
Pyridoxine 5'-phosphate oxidase	<i>pdxH</i>	P5P + O ₂ -> PL5P + H ₂ O ₂
Threonine synthase	<i>thrC</i>	PHT -> 4HLT + PI
Hypothetical Enzyme		4HLT -> PYRDX
Pyridoxine kinase	<i>pdxK</i>	PYRDX + ATP -> P5P + ADP
Hypothetical Enzyme		P5P -> PYRDX + PI
Hypothetical Enzyme		PL5P -> PL + PI
Pyridoxine kinase	<i>pdxK</i>	PL + ATP -> PL5P + ADP
Pyridoxine 5'-phosphate oxidase	<i>pdxH</i>	PYRDX + O ₂ -> PL + H ₂ O ₂
Pyridoxine 5'-phosphate oxidase	<i>pdxH</i>	PL + O ₂ + NH ₃ -> PDLA + H ₂ O ₂
Pyridoxine kinase	<i>pdxK</i>	PDLA + ATP -> PDLA5P + ADP

Hypothetical Enzyme	Reaction	Count
Pyridoxine 5'-phosphate oxidase	$PdxH$ PDLA5P \rightarrow PDLA + PI	0
Serine hydroxymethyltransferase (serine methylase)	$glyA$ PDLA5P + O2 \rightarrow PL5P + H2O2 + NH3	1
Serine hydroxymethyltransferase (serine methylase)	$glyA$ PL5P + GLU \rightarrow PDLA5P + AKG	1
Glutamine fructose-6-phosphate Transaminase	$glmS$ PL5P + ALA \rightarrow PDLA5P + PYR	1
Phosphoglucosamine mutase	$glmM$ F6P + GLN \rightarrow GLU + GAGP	1
N-Acetylglucosamine-1-phosphate-uridylyltransferase	$glmU$ GAGP \leftrightarrow GAIP	0
UDP-N-acetylglucosamine acetyltransferase	$lpxA$ UTP + GAIP + ACCOA \rightarrow UDPNAG + PPI + COA	1
UDP-3-O-acyl-N-acetylglucosamine deacetylase	$lpxC$ C140ACP + UDPNAG \rightarrow ACP + UDPG2AA	1
UDP-3-O-(3-hydroxymyristoyl)glucosamine-acyltransferase	$lpxD$ UDPG2AA \rightarrow UDPG2A + AC	1
UDP-sugar hydrolase	$ushA$ UDPG2A + C140ACP \rightarrow ACP + UDPG23A	1
Lipid A disaccharide synthase	$lpxB$ UDPG23A \rightarrow UMP + LIPX	1
Tetracydisaccharide 4' kinase	$lpxB$ LIPX + UDPG23A \rightarrow UDP + DISAC1P	1
3-Deoxy-D-manno-octulosonic-acid transferase (KDO transferase)	$kdtA$ DISAC1P + ATP \rightarrow ADP + LIPIV	0
3-Deoxy-D-manno-octulosonic-acid transferase (KDO transferase)	$kdtA$ LIPIV + CMPKDO \rightarrow KDOLIPIV + CMP	1
Endotoxin synthase	$htrB$ KDOLIPIV + CMPKDO \rightarrow K2LIPIV + CMP	1
3-Deoxy-D-manno-octulosonic-acid 8-phosphate synthase	$msbB$ K2LIPIV + C140ACP + C120ACP \rightarrow LIPA +2 ACP	2
3-Deoxy-D-manno-octulosonic-acid 8-phosphate phosphatase	$msbA$ PEP + A5P \rightarrow KDOP + PI	1
CMP-2-keto-3-deoxyoctonate synthase	$kdsB$ KDOP \rightarrow KDO + PI	0
ADP-L-glycero-D-mannoheptose-6-epimerase	$kdsB$ KDO + CTP \rightarrow PPI + CMPKDO	1
UDP glucose-1-phosphate uridylyltransferase	$lpcA$ S7P + ATP \rightarrow ADPHEP + PPI	1
Ethanolamine phosphotransferase	$lpcA$ G1P + UTP \rightarrow PPI + UDPG	2
Phosphatidate phosphatase	$lpcA$ PE + CMP \leftrightarrow CDPETN + DGR	0
Diacylglycerol kinase	$lpcA$ PA \rightarrow PI + DGR	0
LPS Synthesis – truncated version of LPS (ref neid)	$lpcA$ DGR + ATP \rightarrow ADP + PA	1
UDP-N-acetylglucosamine-enzolpyruvate transferase	$lpcA$ LPA +3 ADPHEP +2 UDPG +2 CDPETN + 3 CMPKDO \rightarrow	6
UDP-N-acetylglucosamine-enzolpyruvate dehydrogenase	$lpcA$ UDPNAG + PEP \rightarrow UDPNAGEP + PI	1
UDP-N-acetyl muramate-alanine ligase	$murB$ UDPNAGEP + NADPH \rightarrow UDPNAM + NADP	1
UDP-N-acetyl muramoylalanine-D-glutamate ligase	$murC$ UDPNAM + ALA + ATP \rightarrow ADP + PI + UDPNAMA	1
UDP-N-acetyl muramoylalanine-D-glutamate ligase	$murD$ UDPNAMA + DGLU + ATP \rightarrow UDPNAMAG + ADP + PI	1
D-Alanine-D-glutamate ligase	$murE$ UDPNAMAG + ATP + MDAP \rightarrow UNAGD + ADP + PI	1
D-Alanine-D-alanine adding enzyme	$murF$ UNAGD + ATP + AA \rightarrow UNAGDA + ADP + PI	1
Glutamate racemase	$murI$ GLU \leftrightarrow DGLU	1
D-ala:D-ala ligases	$dalAB$ 2 DALA \leftrightarrow AA	2
Phospho-N-acetyl muramoylpentapeptide transferase	$mrAY$ UNAGDA \rightarrow UMP + PI + UNPTDO	1
N-Acetylglucosaminyl transferase	$mrUG$ UNPTDO + UDPNAG \rightarrow UDP + PEPTIDO	1
Arabinose (low affinity)	$araE$ ARA _{Bxt} + HEXT \leftrightarrow ARAB	1
Arabinose (high affinity)	$araFGH$ ARA _{Bxt} + ATP \rightarrow ARAB + ADP + PI	3
Dihydroxyacetone	$DHaxT$ DHaxT + PEP \rightarrow T3P2 + PYR	0

Fructose	<i>fruABF</i>	FRUXt + PEP -> FIP + PYR
Fucose	<i>fucP</i>	FUCxt + HEXT -> FUC
Galactitol	<i>galABC</i>	GLTLxt + PEP -> GLTL1P + PYR
Galactose (low affinity)	<i>galP</i>	GLACxt + HEXT -> GLAC
Galactose (low affinity)	<i>galP</i>	GLCxt + HEXT -> GLC
Galactose (high affinity)	<i>mglABC</i>	GLACxt + ATP -> GLAC + ADP + PI
Glucitol	<i>srI41A2B</i>	GLTxt + PEP -> GLT6P + PYR
Gluconate	<i>gnlST</i>	GLCNxt + ATP -> GLCN + ADP + PI
Glucose	<i>ptsG, crr</i>	GLCxt + PEP -> G6P + PYR
Glycerol	<i>glpF</i>	GLxt -> GL
Lactose	<i>lacY</i>	LCTxt + HEXT -> LCTS
Maltose	<i>malX, crr, male</i>	MLTxt + PEP -> MLT6P + PYR
Mannitol	<i>cmtAB</i>	MNTxt + PEP -> MNT6P + PYR
Mannose	<i>manXYZ, ptsPA</i>	MANxt + PEP -> MAN1P + PYR
Melibiose	<i>melB</i>	MELxt + HEXT -> MEL1
N-Acetylglucosamine	<i>nagE, ptsN</i>	NAG + PEP -> NAGP + PYR
Rhamnose	<i>rhAT</i>	RMNxt + ATP -> RMN + ADP + PI
Ribose	<i>rbsABCD, xyIJI</i>	RIBxt + ATP -> RIB + ADP + PI
Sucrose	<i>scr</i>	SUCxt + PEP -> SUC6P + PYR
Trehalose	<i>treAB</i>	TRExt + PEP -> TRE6P + PYR
Xylose (low affinity)	<i>xytE</i>	XYLxt + HEXT -> XYL
Xylose (high affinity)	<i>xytFG, rbsB</i>	XYLxt + ATP -> XYL + ADP + PI
Alanine	<i>cycA</i>	ALAxt + ATP -> ALA + ADP + PI
Arginine	<i>arg</i>	ARGxt + ATP -> ARG + ADP + PI
Asparagine (low Affinity)	<i>asnQ, arg</i>	ASNxt + HEXT -> ASN
Asparagine (high Affinity)		ASNxt + ATP -> ASN + ADP + PI
Aspartate	<i>gltP</i>	ASPxt + HEXT -> ASP
Aspartate	<i>gltIKL</i>	ASPxt + ATP -> ASP + ADP + PI
	<i>brnQ</i>	BCAAxt + HEXT -> BCAA
	<i>not identified</i>	CYSxt + ATP -> CYS + ADP + PI
	<i>cycA</i>	DALAx + ATP -> DALA + ADP + PI
	<i>cycA</i>	DALAx + HEXT -> DALA
	<i>cycA</i>	DSERxt + HEXT -> DSER
	<i>cycA</i>	GLYxt + HEXT -> GLY
		MDAPxt + ATP -> MDAP + ADP + PI
		GABAxt + ATP -> GABA + ADP + PI
	<i>gabP</i>	GLUxt + HEXT -> GLU
	<i>gltP</i>	GLUxt + HEXT -> GLU
	<i>gltS</i>	GLUxt + HEXT -> GLU

3	Glutamate	<i>gluJKL</i>	GLUxt + ATP -> GLU + ADP + PI
3	Glutamine	<i>ghnHPQ</i>	GLNxt + ATP -> GLN + ADP + PI
4	Glycine	<i>cycA, proVWX</i>	GLYxt + ATP -> GLY + ADP + PI
4	Histidine	<i>hisJMPQ</i>	HISxt + ATP -> HIS + ADP + PI
4	Isoleucine	<i>livJ</i>	ILExt + ATP -> ILE + ADP + PI
6	Leucine	<i>livHKM/livFGJ</i>	LEUxt + ATP -> LEU + ADP + PI
1	Lysine	<i>lysP</i>	LYSxt + HEXT -> LYS
4	Lysine	<i>argT, hisMPQ</i>	LYSxt + ATP -> LYS + ADP + PI
1	Lysine/Cadaverine	<i>caaB</i>	LYSxt + ATP -> LYS + ADP + PI
0	Methionine	<i>metD</i>	METxt + ATP -> MET + ADP + PI
4	Ornithine	<i>argT, hisMPQ</i>	ORNxt + ATP -> ORN + ADP + PI
3	Phenylalanine	<i>aroP/mtr/pheP</i>	PHExt + HEXT -> PHE
4	Proline	<i>putP, proVWX</i>	PROxt + HEXT -> PRO
4	Putrescine	<i>cycA, proVW</i>	PROxt + ATP -> PRO + ADP + PI
5	Proline	<i>putEFHIG</i>	PROxt + ATP -> PTRC + ADP + PI
1	Serine	<i>sdaC</i>	SERxt + HEXT -> SER
1	Serine	<i>cycA</i>	SERxt + ATP -> SER + ADP + PI
4	Serine	<i>potABCD</i>	SPMDxt + ATP -> SPMD + ADP + PI
4	Serine	<i>potABCD</i>	PTRCxt + ATP -> PTRC + ADP + PI
1	Threonine	<i>livJ</i>	THRxt + ATP -> THR + ADP + PI
1	Threonine	<i>ddcC</i>	THRxt + HEXT -> THR
1	Threonine	<i>maB</i>	TRPxxt + HEXT -> TRP
1	Tryptophan	<i>tyrP</i>	TYRxt + HEXT -> TYR
1	Tyrosine	<i>livJ</i>	VALxt + ATP -> VAL + ADP + PI
5	Valine	<i>dppABCDF</i>	DPEPxt + ATP -> DPEP + ADP + PI
5	Dipeptide	<i>oppABCDF</i>	OPEPxt + ATP -> OPEP + ADP + PI
3	Oligopeptide	<i>sapABD</i>	PEPTxt + ATP -> PEPT + ADP + PI
1	Peptide	<i>uraA</i>	URAxxt + HEXT -> URA
1	Uracil	<i>pmuC</i>	NMNxt + HEXT -> + NMN
1	Nicotinamide mononucleotide transporter	<i>codB</i>	CYTSxt + HEXT -> CYTS
1	Cytosine	<i>purB</i>	ADxt + HEXT -> AD
1	Adenine	<i>gpt, hpt</i>	GNxt -> GN
2	Guanine	<i>gpt, hpt</i>	HYXNxt -> HYXN
2	Hypoxanthine	<i>xapB</i>	XTSNxt -> XTSN
1	Xanthosine		XANxt -> XAN
1	Xanthine		ADNxt + HEXT -> ADN
1	G-system		GSNxt + HEXT -> GSN
1	G-system		URxt + HEXT -> URI
1	G-system		

Sulfate transport	7	<i>cysPTUWAZ</i> , <i>s</i>	SLFxt + ATP \rightarrow SLF + ADP + PI
Phosphate transport	4	<i>pstABCS</i>	PIxt + ATP \rightarrow ADP + 2 PI
Phosphate transport	2	<i>pIAB</i>	PIxt + HEXT \leftrightarrow PI
Glycerol-3-phosphate	2	<i>gptT, ugpaBCE</i>	GL3Pxt + PI \rightarrow GL3P
Dicarboxylates	5	<i>dcuAB, dcaA</i>	SUCCxt + HEXT \leftrightarrow SUCC
Dicarboxylates	3	<i>dcaAB, dcta</i>	FUMxt + HEXT \leftrightarrow FUM
Dicarboxylates	3	<i>dcaAB, dcta</i>	MALxt + HEXT \leftrightarrow MAL
Dicarboxylates	3	<i>dcaAB, dcta</i>	ASPxt + HEXT \leftrightarrow ASP
Fatty acid transport	3	<i>fadL</i>	C140xt \rightarrow C140
Fatty acid transport	1	<i>fadL</i>	C160xt \rightarrow C160
Fatty acid transport	1	<i>fadL</i>	C180xt \rightarrow C180
α -Ketoglutarate	1	<i>kgtP</i>	AKGxt + HEXT \leftrightarrow AKG
Na/H antiporter	2	<i>nhaABC</i>	NAXt + \leftrightarrow NA + HEXT
Na/H antiporter	3	<i>chaABC</i>	NAXt + \leftrightarrow NA + HEXT
Pantothenate	3	<i>panF</i>	PNTOxt + HEXT \leftrightarrow PNTO
Sialic acid permease	1	<i>nanT</i>	SLAxt + ATP \rightarrow SLA + ADP + PI
Oxygen transport	1	O2xt	O2 \leftrightarrow O2
Carbon dioxide transport	0	CO2xt	CO2 \leftrightarrow CO2
Urea transport	0	UREAxt + 2 HEXT	UREA \leftrightarrow UREA
ATP drain flux for constant maintenance requirements	0	ATP	\rightarrow ADP + PI
Glyceraldehyde transport	0	GLALxt	\leftrightarrow GLAL
Acetaldehyde transport	0	ACALxt	\leftrightarrow ACAL

Table 2

Comparison of the predicted mutant growth characteristics from the gene deletion study to published experimental results with single and double mutants.

Gene	Glucose (<i>in vivo/in silico</i>)	Glycerol (<i>in vivo/in silico</i>)	Succinate (<i>in vivo/in silico</i>)	Acetate (<i>in vivo/in silico</i>)
<i>aceEF</i>	-/+			
<i>aceA</i>				-/-
<i>aceB</i>				-/-
<i>ackA</i>				+/+
<i>acs</i>				+/+
<i>acn</i>	-/-	-/-	-/-	-/-
<i>cyd</i>	+/+			
<i>cyo</i>	+/+			
<i>eno</i>	-/+	-/+	-/-	-/-
<i>fba</i>	-/+			
<i>fbp</i>	+/+	-/-	-/-	-/-
<i>gap</i>	-/-	-/-	-/-	-/-
<i>gltA</i>	-/-	-/-	-/-	-/-
<i>gnd</i>	+/+			
<i>idh</i>	-/-	-/-	-/-	-/-
<i>ndh</i>	+/+	+/+		
<i>nuo</i>	+/+	+/+		
<i>pfk</i>	-/+			
<i>pgi</i>	+/+	+/+		
<i>pgk</i>	-/-	-/-	-/-	-/-
<i>pgl</i>	+/+			
<i>pntAB</i>	+/+	+/+	+/+	+/+
<i>glk</i>	+/+			
<i>ppc</i>	±/+	-/+	+/+	+/+
<i>pta</i>				+/+
<i>pts</i>	+/+			
<i>pyk</i>	+/+			
<i>rpi</i>	-/-	-/-	-/-	-/-
<i>sdhABCD</i>	+/+			
<i>tpi</i>	-/+	-/-	-/-	-/-
<i>unc</i>	+/+		-/-	-/-
<i>zwf</i>	+/+			
<i>sucAD</i>	+/+			
<i>zwf, pnt</i>	+/+			
<i>pck, mez</i>			-/-	-/-
<i>pck, pps</i>			-/-	-/-
<i>pgi, zwf</i>	-/-			
<i>pgi, gnd</i>	-/-			
<i>pta,acs</i>				-/-
<i>tktA, tktB</i>	-/-			

Results are scored as + or - meaning growth or no growth determined from *in vivo / in silico* data. In 73 of 80 cases the *in silico* behavior is the same as the experimentally observed behavior.